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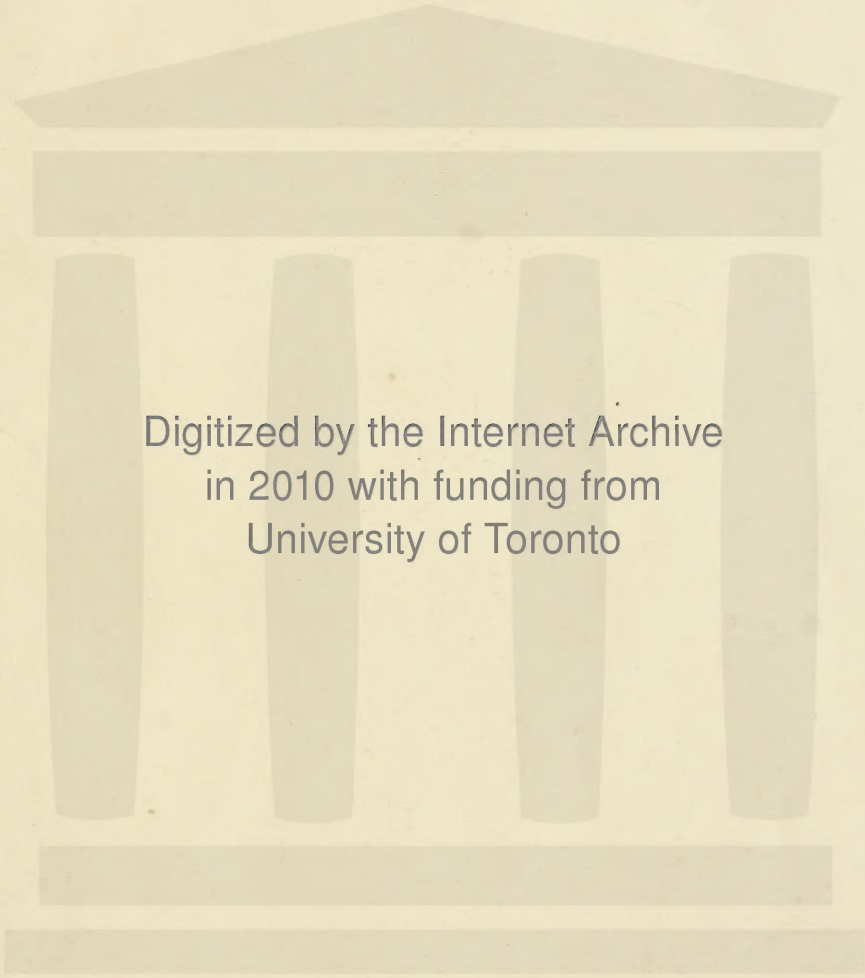

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Annals of Tropical Medicine and Parasitology

Prof. B. Danilewsky

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THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

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CONTENTS

No. 1. March 31, 1913

PAGE

Macfie, J. W. Scott.

Trypanosomiasis of Domestic Animals in Northern Nigeria. Plates I—III ... 1

Stephens, J. W. W.; and Fantham, H. B.

Further Measurements of *Trypanosoma rhodesiense* and *T. gambiense* ... 27

Mayer, T. F. G.

A New Mosquito-proof and Storm-proof House for the Tropics. Plate IV ... 41

Christophers, Major S. R.

Contributions to the Study of Colour Marking and other Variable Characters of *Anophelinae* with Special Reference to the Systematic and Phylogenetic Grouping of Species. Plates V—VIII 45

Blacklock, B.

A Study of the Posterior Nuclear Forms of *Trypanosoma rhodesiense* (Stephens and Fantham) in Rats 101

Balfour, Andrew.

Animal Trypanosomiasis in the Lado (Western Mongalla) and Notes on Tsetse Fly Traps and on an Alleged Immune Breed of Cattle in Southern Kordofan. Plates IX, X 113

Thomson, David.

Sanitation on the Panama Canal Zone, Trinidad and British Guiana. Plates XI, XII, XIII 125

Thomson, John Gordon; and Thomson, David.

The Cultivation of One Generation of Benign Tertian Malarial Parasites (*Plasmodium vivax*) in Vitro, by Bass's Method. Plate XIV 153

Scott, H. Harold.

Fulminating Cerebro-Spinal Meningitis in Jamaica 165

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Full Course begins 6 January. Advanced Course begins 2 June.
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EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the first number of the seventh volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

TRYPANOSOMIASIS OF DOMESTIC ANIMALS IN NORTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A., M.B., CH.B.

WEST AFRICAN MEDICAL STAFF

(*Received for publication* 19 *December*, 1912)

PLATES I—III

Trypanosomiasis is very prevalent amongst domestic animals in Northern Nigeria, and, at any rate in the south-western portions of the Protectorate, accounts not only for a heavy mortality, but also for serious financial losses, due to sickness, forced sales and the slaughtering of stock in unsuitable markets. It is difficult to give any adequate idea of the grave nature of the losses, but some conception of their extent may be gathered from the two following instances:—During the greater part of 1912 I was stationed in Ilorin, a province which, with the exception of one division, is considered not unsuitable for horses. Nevertheless, during the eight completed months of my residence, ten out of the fifteen horses in the possession of Europeans contracted trypanosomiasis, and six died of the disease. An experiment was also made with a view to introducing animal transport into the province. Ten healthy donkeys were selected for this purpose, and sent to carry loads from Ilorin town to Agugi, a village some thirty miles to the east. Within three months all had died of trypanosomiasis. At Zungeru, during the year 1911, twenty-five horses were treated for this disease in the isolation camp, and of these 40 % either died or were so seriously affected that their owners thought it wise to part with them at the small price a native is always willing to pay for a sick animal. At Lokoja the disease is even more serious, and Dr. C. F. Watson has estimated that among the horses treated for tsetse disease there, the rate of dead and incapacitated horses is over 73 %. There can, moreover, be little doubt that the disease is spreading; a fact proved by the additions made from time to time to the list of localities in which the Government declines to compensate officers for the loss of horses which have died from trypanosomiasis contracted in the discharge of official duties.

Difficult as it is to estimate the losses among horses belonging to the small number of Europeans stationed in the Protectorate, it is altogether impossible to ascertain those of the natives. They must, however, be very great, and their magnitude may be gauged by the experiences of the Emirs who attended the Coronation Durbar at Zaria in June, 1911, one of whom is said to have lost 70 per cent. of his horses. Before the British undertook the administration of Nigeria, certain tracts of country known to be unsuitable for horses were sedulously avoided. Europeans, however, are compelled to penetrate these districts in the discharge of their duties, and are followed by the native officials, not only at the sacrifice of their own animals, but also greatly to the detriment of the whole country, since the infected horses returning home, or passing through other districts, spread the disease in every direction.

Besides horses, the cattle, sheep, goats and dogs are also affected. In their long trek to the coast, the herds of cattle from the north carry trypanosomiasis with them, maintaining the infection in old localities and introducing it into new. The herdsmen are quick to detect the symptoms of the disease, and, if they appear in one of their animals, are accustomed to slaughter the beast wherever they may happen to be. The fate of those that sicken is to be butchered by the roadside, and it is a common sight to see a carcase cut up and laid out for sale far away from any native town. Ilorin is situated at a point on the main caravan road where the routes from Kano and Sokoto converge, and the majority of the animals slaughtered there for the market were found on examination to be suffering from trypanosomiasis.

In Table I the results of the examinations of thirty-five of these animals is shown:—

Table I.—Trypanosomes found in the blood of thirty-five sick animals being slaughtered for the Ilorin market

Host	*T. brucei*	*T. vivax*	*T. nanum* or *pecorum*	Double infection
Cattle—Fulani breed	1	18	1	1
Sheep and rams	1	8	1	...
Goats	...	4	...	...
Totals	2	30	2	1

In November, 1911, Sir David Bruce published in No. 31 of the *Sleeping Sickness Bulletin*[1] a list of identifications of fifteen cases collected by me in Northern Nigeria of trypanosomiasis in horses. Since then I have added to these a number of fresh cases, both in horses and other domestic animals, bringing the number up to eighty-eight. An analysis of eighty-six of these cases, in which the infection was natural, is given below (Table II).

TABLE II.—Showing the infecting agent in eighty-six cases of naturally acquired trypanosomiasis in domestic animals in Northern Nigeria

Host	*T. brucei*	*T. vivax*	*T. nanum* or *pecorum*	*T. theileri*	Double infections
Horse	14	18	8	...	3
Donkey	2	2	...	...	...
Cattle—					
Fulani breed	1	18	1	...	1
Dwarf breed	...	2	...	1	...
Sheep and ram	1	8	1	...	...
Goat	...	4	...	...	...
Dog	...	1	...	...	...
Totals ...	18	53	10	1	4

As it was impossible in Northern Nigeria to carry out systematic series of experiments on animals or to attempt cross immunity tests, the morphological characters of the trypanosomes found in blood-films had to be relied on for identification. By this means it was, of course, impossible to distinguish between the more closely allied species. *T. theileri* was observed once, but with this exception the trypanosomes detected fell naturally into three main groups, and, as individuals of each type had previously been examined by Sir David Bruce, his identifications have been adopted.[2]

*T. brucei.** In twenty cases of natural infection trypanosomes were found which closely corresponded with the type described and figured by Sir David Bruce as *T. brucei*.[3] In four cases (three horses and one donkey) posterior nuclear forms were detected, and

* This trypanosome is probably that for which Prof. Stephens and Dr. Blacklock have recently proposed the name *T. ugandae*. (Proc. Roy. Soc., B, Vol. LXXXVI, pp. 187-191).

in guinea-pigs and rats inoculated from three of these cases they appeared at certain stages of the disease in relatively large numbers. Posterior nuclear forms have been described in *T. equiperdum*, *T. pecaudi* and *T. brucei*, as well as in *T. rhodesiense*. Their occurrence in these cases of trypanosomiasis in Nigeria does not therefore assist in precise identification. Sleeping sickness, however, is either altogether absent or quite uncommon at Zungeru and Ilorin, where the majority of my cases were found. In a number of films the long forms of the parasite were observed to have their posterior extremities peculiarly blunt and almost rectangular. No attempt was made to plot a curve to represent the variation in size of this trypanosome. Great variations were observed from day to day in the relative percentages of short and long forms, as is indicated in the tables given below, where the different forms were enumerated in the case of a horse, and in those of a rat and a guinea-pig inoculated with his blood. (See table on page 5.)

The disease set up by these trypanosomes appears to be extremely fatal. Of the eleven horses and two donkeys suffering from this form of trypanosomiasis of which I have complete records none recovered. In horses the disease seems to occur in two forms, some cases end fatally in two to four weeks, others linger on for as many months. All the cases in which posterior nuclear forms were observed were of the acute type, death occurring within a month of the onset of the disease.

The general symptoms in horses were fever, emaciation, and more or less pronounced oedema of the legs, belly and scrotum. The appetite was irregular, but was often ravenous almost up to the hour of death. In the later stages restlessness was a distressing feature. Inflammation of the eyes was observed in two cases out of fourteen, and a greenish discharge from the nose, coughing up of mucus, and a herpetiform eruption on the upper lip occurred each in one case. The animals presented a dejected appearance in the early stages of the disease, their heads drooped, they dragged their hind legs in walking, moved slowly and with difficulty and were apt to stumble. They were notably insensitive to pain, as for instance, to the prick on the ear necessary to obtain blood for examination. Finally they became so weak and wasted that they could not rise.

Natural infections with trypanosomes of this type were found in the horse, donkey, Fulani cattle, and sheep. The parasite was also successfully transmitted by inoculation to a cow of the dwarf breed found in pagan districts of Nigeria. (See page 10.)

Date, 1912	Horse (No. 35)		Rat		Guinea-pig	
	Forms with long, free flagella	Stumpy and intermediate forms	Forms with long, free flagella	Stumpy and intermediate forms	Forms with long, free flagella	Stumpy and intermediate forms
July 8 ...	24%	76%	...	...	...	...
,, 9 ...	42%	58%	...	...	...	...
,, 10 ...	...	...	Rat injected from horse. (Trypanosomes first appeared in the blood on July 16)		Guinea-pig injected from horse. (Trypanosomes first appeared in the blood on July 18)	
,, 13 ...	29%	71%	...	...	...	...
,, 16 ...	Trypanosomes very scanty		89%	11%	...	...
,, 17 ...	...	...	98%	2%	...	...
,, 18 ...	...	...	52%	48%	15%	85%
,, 19 ...	...	...	15%	85%	40%	60%
,, 20 ...	74%	26%	10%	90%	56%	44%
,, 21 ...	...	...	8%	92%	16·5%	83·5%
,, 27 ...	6%	94%	88%	12%	Died July 21	
,, 28 ...	Died		...	...		
,, 29 ...			...	...		
,, 30 ...			58%	42%		
Aug. 1 ...			85%	15%		
,, 4 ...			69%	31%		
,, 5 ...			Died			

T. vivax. By far the most common form of trypanosome in my series of cases was one presenting the morphological features of *T. vivax*. It occurred in fifty-six of my eighty-six cases, and in fifty-three cases was the only trypanosome present, being but once associated with *T. brucei* and twice with *T. nanum* or *pecorum*.

This parasite was found in the following hosts:—Horse, donkey, Fulani cattle, dwarf cattle of Nigeria, sheep, goat and dog. From the fact that it was found in the dog, it is probable that this trypanosome is the parasite to which Ziemann[4] gave the name *T. vivax*, and not the allied *T. cazalboui* described by Pecaud as occurring in Dahomey in horses. For Laveran and Mesnil say: 'Le fait que *Tr. Cazalboui* ne peut être inoculé ni au singe, ni au chien, ni aux petits rongeurs, permet de distinguer facilement ce trypan. des espèces qui s'en rapprochent au point de vue morphologique, mais qui sont pathogènes pour ces animaux.'[5]

In horses the disease set up by this parasite seems to be mild in Northern Nigeria. Of the fifteen cases of which I have complete records only one died, whereas fourteen recovered. The general symptoms were fever, emaciation and oedema of the legs, belly and scrotum, sometimes well marked, at other times scarcely perceptible. The animals presented a dejected appearance, moved awkwardly and reluctantly, and appeared as though tired. In severe cases they were somewhat insensitive to pain. The appetite was always good. In one case cough was a symptom, and in another nasal catarrh. No affections of the eyes were observed. Three cases were met with in donkeys, and of these two died and one recovered. The case of one of those that died was, however, complicated by a concurrent infection with *T. brucei*. Eighteen cases were found amongst Fulani cattle. Most of them were animals treking down towards Lagos from Kano or Sokoto which had sickened on the road, and were therefore about to be butchered for the Ilorin market. Their native owners believed them to be dying, and were therefore having them slaughtered so as to anticipate death; but, beyond this assertion, I have no data from which to gauge the mortality from the disease in this breed of cattle. Both the dwarf cattle which came under my notice suffering from *T. vivax*, however, recovered. Similarly the eight sheep and rams I studied were animals about to be slaughtered so as to anticipate death, and the same remark applies to the four goats. The one dog in which *T. vivax* was found died.

T. nanum or *pecorum*. In a few cases small trypanosomes 9-12μ in length without free flagella were found in blood-films, and these have been identified as *T. nanum* or *pecorum*. 'It will be

remembered the name *T. pecorum* was introduced by Bruce to cover the group of trypanosomes of which the *T. dimorphon* of Laveran and Mesnil and *T. congolense* are members, and that *nanum* is only distinguishable from *pecorum* by animal experiments.'[6]

Trypanosomes of this type were met with in thirteen cases, in ten alone and in three associated with *T. vivax* or *T. brucei*. Ten of these cases were horses, two Fulani cows, and one a ram. Of the ten cases in horses one had a concurrent infection with *T. vivax* and another with *T. brucei*, leaving eight uncomplicated cases, of which one died, three recovered, and the remaining four had unknown issues.

The number of cases of this variety of trypanosomiasis that came under my notice does not, therefore, enable me to give any general account of the symptoms of the disease in Northern Nigeria. It may, however, be noted that oedema was present only to a slight extent in the cases seen, although lachrymal and nasal discharges were relatively more frequent than in infections with either *T. vivax* or *T. brucei*.

T. theileri. *T. theileri* was observed once in a cow of the dwarf breed found in Nigeria. The animal was feverish for a few days and wasted considerably, but soon recovered from the infection.

The period when trypanosomiasis is most common is undoubtedly the rainy season, which corresponds to the time during which the tsetse flies, which in the dry season retire to the banks of the streams and rivers, are most widely distributed over the country. The accompanying chart of the rainfall and the number of admissions of horses to the isolation camp at Zungeru for the year 1911 illustrates this point, although the number of cases dealt with is too small to form a reliable index. A better proof is afforded by the fact that the natives congratulate themselves at the end of the rainy season that those of their horses which have withstood the disease so far will be safe until the rains recommence. The dry season is also the time of year selected by the herdsmen of the north to undertake their long trek south with their cattle and sheep.

The province of Ilorin in Northern Nigeria is peculiar, inasmuch as, whilst *G. palpalis* and *G. tachinoides* are distributed all over it, tsetse flies of the *morsitans* group—*G. submorsitans* and *G. longipalpis*—are restricted to one, the Patigi or eastern, division. In

my series of cases of trypanosomiasis collected at Ilorin, twenty-four were animals that had never been into the Patigi division, and of these fourteen were infected with *T. vivax*, six with *T. brucei* and four with *T. nanum* or *pecorum*. The Patigi division is shunned by all herdsmen, and it is generally recognised that horses can neither live there nor be taken into the district without contracting tsetse disease. Two horses that had lived for years in Ilorin town were taken to Patigi during my last tour of service.

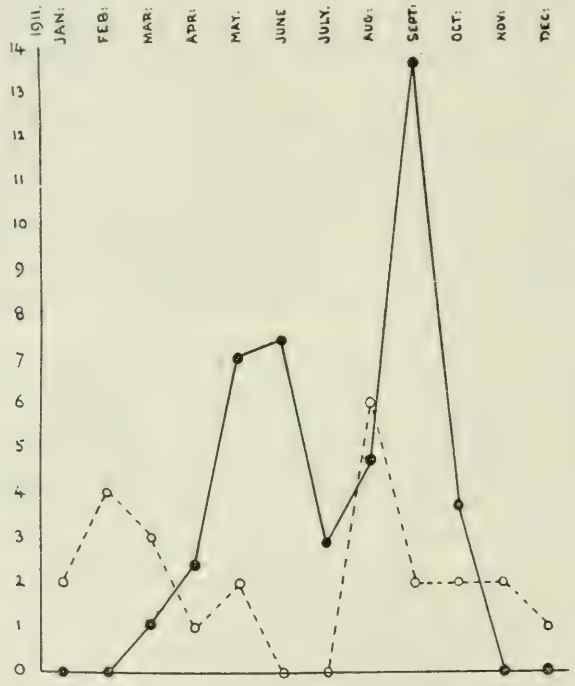

Chart showing the rainfall in inches (continuous line) and the number of cases of trypanosomiasis of horses admitted to the isolation camp (dotted line) at Zungeru during 1911.

Both returned infected with *T. brucei*, and both died shortly after wards. In this connexion it should be mentioned that Bruce concludes that, in Uganda, 'The carrier of *Trypanosoma vivax* is probably *Glossina palpalis*,'[7] and that *G. morsitans* is known to transmit *T. brucei*. It is possible that in the native towns and European stations flies of the genus *Stomoxys*, which abound in these places, may play a part in the transmission of trypanosomiasis. The horse (No. 42), for instance, had not been within

two miles of any spot known to be haunted by tsetse flies for five weeks previous to the onset of his symptoms, and it is practically certain that during this period he could not have been bitten by tsetse flies. He was, however, tormented by *Stomoxys* flies, which were exceedingly common at the time. Both *S. nigra* and *S. calcitrans* have been taken at Ilorin.

Treatment. The treatment usually adopted in Northern Nigeria for all cases of trypanosomiasis in horses is the administration of arsenious acid and perchloride of mercury by the mouth. This is the routine treatment adopted at the isolation camps at Zungeru and Lokoja. The doses usually employed are nine grains of the arsenic and three ounces of a 1 in 1,000 solution of the perchloride of mercury daily, divided into three doses; but I have also tried much larger doses, administered for a few days at a time only, in the hope that the sudden shock of arsenic might eliminate the trypanosomes before they had time to react to the drug, and with a view to avoiding the cumulative action on the host that has certainly sometimes proved fatal. It is doubtful whether this treatment has any beneficial results. In my experience it has not saved a single animal infected with *T. brucei*, and it is impossible to judge of its action on *T. vivax*, as this form of trypanosomiasis is as a rule not fatal, the animals recovering even when untreated. At one time it was asserted that such treatment accounted for the cure of 50 % of the cases at Zungeru. This, however, was before it was known that half the trypanosomiasis there was due to *T. vivax*, and was capable of spontaneous cure. The higher mortality recorded at Lokoja is probably accounted for by the larger percentage of cases of trypanosomiasis due to *T. brucei* which occur at that station.

Intravenous injections of antimony have met with no greater success in trypanosomiasis due to *T. brucei*. Organic arsenical compounds have been used in only a few cases, the high cost of the injections and the technical difficulties of administration standing in the way of the general use of these preparations until it is certain, not only that a cure will result, but also that immunity against future attacks will be established.

Arsenic and mercury have been used as prophylactics also, but without success.

No immunity follows an attack of trypanosomiasis, reinfections with the same or a different species of trypanosome being met with. The dwarf cow (No. 48) in my series was infected successively with *T. theileri*, *T. vivax* and *T. brucei*, and finally died of the last form of trypanosomiasis. The donkey (No. 47), after recovering from an infection with *T. vivax*, was infected by injection with *T. brucei*, and succumbed to the disease.

Treatment of any sort is, however, applicable only to Government stock and horses in the possession of Europeans. The great majority of cases, occurring in animals which are the property of natives, cannot be brought under treatment. It might be possible, nevertheless, to limit the spread of the disease by the establishment of isolation camps, and much might be effected by systematic clearing along the roads. The main caravan routes especially should be cleared, and inducement offered to the natives to farm the land on either side of the roads.

Dwarf cattle. In certain districts in which the Fulani cattle do not live a few dwarf cattle are found, which, because they occur in pagan country, are often referred to as 'pagan cattle.' In Ilorin they are kept in all the districts occupying the south-eastern corner of the province, from Ofa Ora to Ejiba on the north, and extending west along the southern boundary as far as Oke Awra. Dr. Foy, who made some interesting observations and experiments on a bull of this breed, thus described his physical characters. 'The body frame,' he says, 'was thick-set and broad and comparatively long for its height, the legs thick and short, the neck thick and short, the head short, and the horns short.'[8] The accompanying photograph of a bull and cow brought from Awtun will help to give an accurate idea of the appearance of these singular animals (Pl. III, fig. 5).

The most remarkable point about these dwarf cattle, which are bred for slaughter only and are not used for milking, is that they live in districts in which the Fulani cattle cannot exist. For this reason it has been conjectured that they have acquired a high degree of immunity to trypanosome infections. Dr. Foy, who, as already stated, experimented on a bull of this breed, concluded:—

'(1) That a certain breed of cattle found in pagan districts possess a high degree of natural immunity in that they may harbour the trypanosome in the blood and yet keep in good condition and

show no signs of the disease, nor do they die from the infection so long as their environments are favourable. These environments are a free life, with ample food, especially plenty of green grass. Confinement, poor feeding, and hard exercise, tend to make the disease manifest itself clinically.

'(2) That such domesticated cattle may act as a reservoir of infection since the blood may prove infective at such times when clinical symptoms manifest themselves, although the trypanosome may not be found on making a microscopical examination.

'(3) That the subcutaneous injection of 26 c.c. of serum obtained from the blood of one of these cattle when manifesting no clinical symptoms of the disease did not prove infective when inoculated into a calf, nor did it act as a prophylactic when the calf was exposed to natural infection subsequently.'

He adds, in another part of the same paper, 'all strains of trypanosomes used or met with in the work were of the *T. brucei* type.'

At Ilorin I was able to carry out only two experiments with these cattle, but as the subject requires careful investigation, and promises to provide results of practical importance, I should, perhaps, place them on record here. On May 11th, 1912, two of these dwarf cattle (a cow and a bull) arrived at Ilorin from Awtun. As they had treked up all the way by road, they arrived in rather poor condition, but seemed, on the whole, to have stood the unusual exertion wonderfully well. On May 13th I made a thorough examination of the blood of each without detecting any trypanosomes, and four days later injected a rat and a guinea-pig each with 1 c.c. of blood taken from the cow. Neither of these animals developed any symptoms of disease, and, although they were kept under close and constant observation for four weeks, trypanosomes were never seen in their blood. The subsequent history of the cattle is given below:—

Dwarf Bull (No. 50)

May 11th, 1912.—Arrived at Ilorin.

May 13th, 1912.—Blood examination—negative.

August 30th, 1912.—Trypanosomes (*T. vivax*) in the blood. Bull looks well. Untreated.

September 15th, 1912.—Blood examination—negative. Bull, however, looks ill.

September 19th, 1912.—Bull died of anthrax. No trypanosomes found in the blood nor in smears made from the organs after death.

Dwarf Cow (No. 48)

May 11th, 1912.—Arrived at Ilorin.

May 13th, 1912.—Blood examination—negative.

May 17th, 1912.—Blood examination—negative. Subcutaneous injections of the cow's blood made into a rat and a guinea-pig.

Rat under observation twenty-eight days, but never showed trypanosomes.

Guinea-pig under observation twenty-eight days but never showed trypanosomes.

August 30th, 1912.—Cow feverish and emaciated, no oedemas. Trypanosomes (*T. theileri* and *T. vivax*) present in the blood. Untreated.

September 7th, 1912.—Trypanosomes still present in the blood. Subcutaneous injection of two c.c of the cow's blood into a guinea-pig.

Guinea-pig under observation nineteen days. Accidentally killed. Never showed trypanosomes.

September 18th, 1912.—Blood examination—negative. One c.c. of the cow's blood injected subcutaneously into a rat.

Rat under observation twenty-two days but never showed trypanosomes.

September 27th, 1912.—Cow looks well, and her blood has been free from trypanosomes since September 15th. Two c.c. of blood from donkey (No. 45), heavily infected with *T. brucei*, injected into cow subcutaneously.

October 8th, 1912.—Trypanosomes numerous (*T. brucei*). The blood was negative up to October 5th.

November 3rd, 1912.—Cow died. Trypanosomes still present in the blood but scanty.

Both animals developed a natural infection with *T. vivax*, and apparently recovered, for they ceased to show trypanosomes in films made from the peripheral blood. The cow in addition had a concurrent infection with *T. theileri*. The cow was then successfully infected by injection of blood from a donkey heavily infected with *T. brucei*, and, although the parasites soon became scarce in her blood, she died five weeks after the injection still showing a few trypanosomes in the peripheral blood.

It would be rash to draw definite conclusions from such experiments, but so far as they go they do tend to show that this breed of cattle, like horses, possesses an immunity to *T. vivax*, inasmuch as although these parasites may appear in their blood they do not produce a rapidly fatal disease. The immunity does not appear, however, to extend to *T. brucei*. The question then arises how do these cattle live in districts where the Fulani herdsmen dare not take their cattle to graze? The dwarf cattle are generally to be found in the immediate vicinity of villages, and unlike the Fulani cattle do not wander widely over the country in search of pastures. It was thought possible, therefore, that they might thus escape the attacks of *G. submorsitans*, the species of tsetse fly whose distribu-

tion (as already pointed out) is coextensive with the areas habitually shunned by the Fulani herdsmen. In consequence, collections of flies were obtained at Odo Okeri, Eri, Oro and Oke Onio, from spots where the cattle were actually grazing. In none of these collections was *G. submorsitans* present, although both *G. palpalis* and *G. tachinoides* were taken. I have to thank Mr. T. A. G. Budgen for very kindly superintending the work of a collector who was sent with him for this purpose. I can only hope that at some future date, and under more favourable conditions, it may be possible to make a thorough investigation of this interesting subject.

In conclusion, I would like to express my thanks to Mr. E. C. Duff for affording me an opportunity of examining the dwarf breed of cattle; to Dr. C. F. Watson and Dr. W. Morrison for very kindly sending me blood-films from Lokoja; to Dr. G. R. Twomey, who relieved me at Ilorin, for following the last stages of my experiments; and to Serjt. Moore for the careful record kept by him of the cases of trypanosomiasis occurring at Zungeru.

A tabular synopsis of the cases of trypanosomiasis in domestic animals, collected in Northern Nigeria, is appended.

SYNOPSIS OF CASES OF TRYPANOSOMIASIS OF DOMESTIC ANIMALS

COLLECTED IN NORTHERN NIGERIA

No.	Place	Host	Date	Identification	Result	Remarks
1	Minna	Horse	Aug., 1910	*T. vivax* *	Recovered	Mild case, no oedema.
2	Bida	,,	Sept., 1910	*T. nanum* or *pecorum**	,,	
3	Zungeru	,,	Nov., 1910	*T. vivax* *	,,	
4	,,	,,	,,	*T. vivax* *	(?)	Died of intercurrent disease.
5	,,	,,	Jan., 1911	*T. brucei* *	Died	Ill 2 months. Latterly extremely emaciated, refusing food and water, unable to rest and constantly walking round and round in a small circle with the head bent almost down to the ground.
6	Benue R. near Umaisha	,,	Dec., 1910	*T. brucei* *	(?)	
7	Ankpa	,,	Jan., 1911	*T. vivax* *	(?)	A severe case. A large swelling formed under the throat, burst in a few days, and discharged large quantities of watery fluid.
8	Zungeru	,,	,	*T. vivax* *	Recovered	
9	,,	,,	,,	*T. brucei* *	Died	Ill 7 weeks.
10	,,	,,	Feb., 1911	*T. vivax* *	Recovered	
11	,,	,,	,,	*T. vivax* *	,,	
12	,,	,,	Mar., 1911	*T. brucei* *	Died	Also microfilariae. Ill 5 months.
13	,,	,,	May, 1911	*T. vivax* *	Recovered	
14	,,	,,	,,	*T. vivax* *	,,	
15	,,	,,	,,	*T. vivax* *	,,	

* Identifications by Sir David Bruce.

Synopsis of Cases of Trypanosomiasis of Domestic Animals, Collected in Northern Nigeria.—*continued.*

No.	Place	Host	Date	Identification	Result	Remarks
16	Lokoja	Horse	Oct., 1911	*T. brucei* †	Died	Had recovered from a previous attack of trypanosomiasis.
17	,,	,,	,,	*T. vivax* †	Recovered	
18	,,	,,	,,	*T. brucei* †	Died	'Ill for months.'
19	,,	,,	Nov., 1911	*T. brucei* †	(?)	'Ill for months.'
20	,,	,,	,,	*T. vivax* †	Recovered	
21	,,	,,	,,	*T. nanum* or *pecorum*	(?)	Sold to a native.
22	,,	,,	Mar., 1912	*T. nanum* or *pecorum*	Died	
23	,,	,,	Feb., 1912	*T. vivax*	,,	'Very marked oedema of joints, legs, abdomen and testicles.'
24	Zungeru	,,	Nov., 1911	*T. brucei*	,,	
25	,,	,,	Dec., 1911	*T. vivax*	Recovered	
26	,,	,,	Jan., 1912	*T. vivax*	(?)	
27	,,	,,	,,	*T. nanum* or *pecorum*	Recovered	But see No. 33. Lachrymal and nasal discharges. Guinea-pig injected with negative result.
28	,,	,,	,,	*T. nanum* or *pecorum*	(?)	Sold to a native.
29	,,	,,	,,	*T. nanum* or *pecorum*	(?)	
30	,,	,,	Feb., 1912	*T. brucei*	(?)	

† Identifications by Dr. Bladlock, The Runcorn Research Laboratory.

Synopsis of Cases of Trypanosomiasis of Domestic Animals. Collected in Northern Nigeria.—*continued.*

No.	Place	Host	Date	Identification	Result	Remarks
31	Zungeru	Horse	Feb., 1912	*T. nanum* or *pecorum*	(?)	
32	Ilorin	,,	,,	*T. vivax*	Recovered	Also microfilariae present.
33	,,	,,	April, 1912	*T. nanum* or *pecorum* and *T. vivax*	Died	Same horse as No. 27. Ill 19 days. Very marked oedema subsiding latterly leaving the pony a mere bag of bones. Profuse diarrhoea.
34	Ilorin	,,	June, 1912	*T. vivax*	Recovered	Mild infection.
35	Offa	,,	July, 1912	*T. brucei*	Died	Pl. I, fig. 1. Microfilariae also present. Ill 30 days. Posterior nuclear forms present; oedema slight. In rat incubation 6 days, death on 26th day after injection. In guinea-pig incubation 8 days, death on 11th day after injection.
36	Ilorin	,,	Aug., 1912	*T. nanum* or *pecorum*	Recovered	Also microfilariae present.
37	Patigi	,,	,,	*T. brucei*	Died	Posterior nuclear forms present. Ill 11 days.
38	Ilorin	,,	,,	*T. brucei*	,,	Microfilariae detected in pony's blood last April. Ill 22 days. Eye symptoms present.
39	,,	,,	,,	*T. nanum* or *pecorum* and *T. brucei*	(?)	Still ill on October 19th. *T. brucei* very scanty. A very young pony.
40	,,	,,	,,	*T. vivax*	Recovered	
41	,,	,,	Sept., 1912	*T. vivax* and *T. brucei*	Died	Ill about 2 months.

SYNOPSIS OF CASES OF TRYPANOSOMIASIS OF DOMESTIC ANIMALS. COLLECTED IN NORTHERN NIGERIA.—*continued.*

No.	Place	Host	Date	Identification	Result	Remarks
42	Ilorin	Horse	Oct., 1912	*T. brucei*	Died	Pl. I, fig. 2. Possibly infected directly by Stomoxys from donkey No. 45 or No 47. Posterior nuclear forms present. Well marked oedema. Ill 2 weeks. In guinea-pig incubation 19-23 days, death 42nd day.
43	Patigi	,,	,,	*T. brucei*	,,	Ill about 3 weeks.
44	Ilorin	Donkey	Aug., 1912	*T. brucei*	,,	Pl. II, fig. 3. Posterior nuclear forms present. Slight oedema. Ill about 3 weeks. In rat incubation 10 days, still alive on 34th day. In guinea-pig incubation 10 days, died on 58th day.
45	,,	,,	Sept., 1912	*T. brucei*	,,	Pl. II, fig. 4. Ill about 8 weeks.
46	,,	,,	,,	*T. vivax*	,,	
47	,,	,,	,,	*T. vivax*	Recovered	
47A	,,	,,	Oct., 1912	*T. brucei*	Died	Injected from donkey No. 45. Died 24 days after injection. Little or no oedema.
48	,,	Dwarf cow	Aug., 1912	*T. theileri*	Recovered	Pl. III, fig. 5.
49	,,	,,	Sept., 1912	*T. vivax*	,,	
49A	,,	,,	Oct., 1912	*T. brucei*	Died	Injected from donkey No. 45. Died 37 days after injection.
50	,,	Dwarf bull	Aug., 1912	*T. vivax*	Recovered	
51	,,	Fulani cow	Sept., 1912	*T. nanum* or *pecorum*	Slaughtered	Treking South.
52	,,	,,	,,	*T. vivax*	,,	,, ,,
53	,,	,,	,,	*T. vivax*	,,	,, ,,
54	,,	Fulani bull	,,	*T. vivax*	,,	,, ,,

Synopsis of Cases of Trypanosomiasis of Domestic Animals. Collected in Northern Nigeria.—*continued.*

No.	Place	Host	Date	Identification	Result	Remarks
55	Ilorin	Fulani cow	Sept., 1912	*T. vivax*	Slaughtered	Treking South from Kano.
56	,,	Fulani bull	,,	*T. vivax*	,,	,, ,, ,,
57	,,	,,	,,	*T. vivax*	,,	,, ,, ,,
58	,,	Fulani cow	,,	*T. vivax*	,,	,, ,, ,,
59	,,	Fulani bull	,,	*T. vivax*	,,	,, ,, ,,
60	,,	,,	,,	*T. vivax*	,,	,, ,,
61	,,	,,	,,	*T. vivax*	,,	,, ,, ,,
62	,,	,,	,,	*T. vivax*	,,	,, ,, ,,
63	,,	,,	,,	*T. vivax*	,,	,, ,,
64	,,	,,	,,	*T. vivax*	,,	Treking South from Kano. Ill 10 days.
65	,,	,,	,,	*T. vivax*	,,	,, ,, ,, ,, ,,
66	,,	Fulani cow	,,	*T. nanum* or *pecorum* and *T. vivax*	Sent to Ibadan to be slaughtered	Ilorin cow.
67	,,	,,	,,	*T. vivax*	Slaughtered	Treking South from Kano.
68	,,	,,	Oct., 1912	*T. brucei*	,,	,, ,, ,, (Jebba route). Posterior nuclear forms present.
69	,,	,,	,,	*T. vivax*	,,	,, ,, from Hadeija (Patigi route).
70	,,	,,	,,	*T. vivax*	,,	,, ,, from Hausa country (Jebba route).
71	,,	,,	,,	*T. vivax*	,,	,, ,, ,, ,, ,,

No.	Place	Host	Date	Identification	Result	Remarks
72	Ilorin	Sheep	Sept., 1912	*T. vivax*	Slaughtered	
73	,,	Ram	,,	*T. brucei*	,,	Long forms 21 μ, stumpy 13-14 μ
74	,,	,,	,,	*T. vivax*	,,	Treking South from Kano.
75	,,	,,	,,	*T. vivax*	,,	,, ,, from North.
76	,,	Sheep	,,	*T. vivax*	,,	,, ,, from Kano.
77	,,	Ram	,,	*T. vivax*	,,	,, ,,
78	,,	,,	,,	*T. vivax*	,,	,, ,, ,,
79	,,	,,	,,	*T. vivax*	,,	,, ,, ,, (Patigi route).
80	,,	Sheep	Oct., 1912	*T. vivax*	,,	From Alapa, Ilorin:
81	,,	Ram	,,	*T. nanum* or *pecorum*	,,	Treking South from Kano (Jebba route).
82	,,	Goat	Sept., 1912	*T. vivax*	,,	
83	,,	,,	,,	*T. vivax*	,,	
84	,,	,,	Oct., 1912	*T. vivax*	,,	Ilorin goat.
85	,,	,,	,,	*T. vivax*	,,	,, ,,
86	,,	Dog	Sept., 1912	*T. vivax*	Died	

SUMMARY

1. Trypanosomes presenting the morphological characters of *T. brucei*, *T. vivax*, *T. nanum* or *pecorum*, and *T. theileri* have been found in Northern Nigeria in the blood of domestic animals; *T. brucei* in the horse, donkey, Fulani cattle, dwarf cattle and sheep; *T. vivax* in the horse, donkey, Fulani cattle, dwarf cattle, sheep, goat and dog; *T. nanum* or *pecorum* in the horse, Fulani cattle and sheep; and *T. theileri* in the dwarf cattle.

2. *T. vivax* is the most common form at any rate in the south-western portions of the Protectorate, being present in fifty-five out of eighty-four cases collected in Ilorin province, the Niger province, and at Lokoja.

3. Of twenty Fulani cattle treking down towards the coast from the provinces of the north, and found to be suffering from trypanosomiasis, eighteen harboured *T. vivax*.

4. In horses *T. vivax* produces a much less serious disease than *T. brucei*. Of fifteen cases infected with *T. vivax*, of which the records are complete, fourteen recovered. Of eleven cases infected with *T. brucei* none recovered.

5. The dwarf breed of cattle found in certain tsetse-haunted districts of Northern Nigeria, and credited with a natural immunity to trypanosomiasis, while apparently recovering from infections with *T. vivax*, succumb to *T. brucei*.

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EXPLANATION OF PLATE I

Fig. 1. Horse (No. 35). Trypanosomiasis (*T. brucei*). Ilorin, July, 1912.

Fig. 2. Horse (No. 42). Trypanosomiasis (*T. brucei*). Ilorin, October, 1912.

EXPLANATION OF PLATE II

Fig. 3. Donkey (No. 44). Trypanosomiasis (*T. brucei*). Ilorin, August, 1912.

Fig. 4. Donkey (No. 45). Trypanosomiasis (*T. brucei*). Ilorin, September, 1912.

EXPLANATION OF PLATE III

Fig. 5. Dwarf cattle. Ilorin, May, 1912.

FURTHER MEASUREMENTS OF *TRYPANOSOMA RHODESIENSE* AND *T. GAMBIENSE*

BY

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In our former paper (May, 1912) on this subject, we came to the conclusion that it was advisable in measuring trypanosomes to confine our observations to those from a single animal, for example, a rat, as although it could not be definitely proved that the size of the trypanosome varied in different animals, yet it appeared likely from the general consensus of opinion that this might be so. From the statistical side the criticism has also been brought that samples of twenty at any particular time are too small. In our present series of measurements therefore, we have, as far as possible, met these objections by measuring always from a single animal, a rat, and by measuring one hundred trypanosomes each day for the first ten days of the infection. We may here briefly repeat our method, as it has been subjected to some criticism.

1. We project the trypanosomes on a screen in a dark room and trace them, instead of drawing them with a camera lucida. It has been objected that this method cannot be used in the wilds of Africa, but we never stated that it could or should, and that is no reason why we should not use it in a laboratory. Our critics might as well object to our using electric light.

2. We measure the trypanosomes by means of the 'tangent line' method. We believe that as this method is the most accurate known—our critics have not attempted to deny this—we are again amply justified in preferring it to the less accurate compass method, even though the difference may be only 1 or 2 μ. It seems to us hardly a matter for argument that if it is worth measuring 1,000 trypanosomes at all, it is worth doing so accurately, especially if length is to be considered a criterion of specificity.

We believe that it is important to give the actual data as to measurements as Bruce does, and not simply averages, as the actual data are necessary for a closer analysis than averages permit. We give first, then, the fundamental data for each trypanosome (*T. rhodesiense* and *T. gambiense*) and the tables compiled from them. We shall subsequently make a comparative analysis of the two sets of figures.

Table I.—Distribution in respect to length of 1,000 non-dividing individuals of *Trypanosoma rhodesiense* from a single white rat.

		In Microns																						Averages	
		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	of each 20	of each 100
1st Day ...	1	...	...	...	...	...	...	...	1	2	2	1	1	3	3	4	1	2	...	...	...	...	...	...	26·0	
	2	...	...	...	...	...	1	2	1	2	2	3	1	5	...	3	...	...	...	...	...	...	...	...	24·05	
	3	...	...	...	...	...	1	1	...	2	2	...	2	3	2	3	3	1	...	...	...	...	...	...	25·6	25·14
	4	...	...	...	...	2	1	2	...	1	2	2	1	4	1	1	3	...	...	...	...	...	...	...	24·1	
	5	...	...	...	...	...	...	...	...	1	3	...	6	4	1	2	1	...	2	...	...	...	...	...	25·95	
2nd Day ...	6	...	...	...	...	...	...	1	1	1	3	...	3	2	1	5	3	...	...	...	...	...	...	...	25·65	
	7	...	...	...	...	...	...	...	...	...	2	3	1	5	4	4	1	...	...	...	...	...	...	...	26·1	
	8	...	...	1	2	3	3	2	3	...	...	1	...	3	...	2	...	...	...	...	...	...	...	...	21·1	22·98
	9	...	...	1	...	5	2	1	4	3	1	1	...	1	...	...	...	1	...	...	...	...	...	...	20·85	
	10	...	...	1	...	2	2	4	3	2	2	...	3	1	...	...	...	...	...	...	...	...	...	...	21·2	
3rd Day ...	11	...	...	...	...	...	1	2	1	3	5	1	3	3	1	...	...	...	...	...	...	...	...	...	23·25	
	12	1	...	...	1	...	2	1	3	2	2	3	2	1	2	...	...	...	...	...	...	...	...	...	22·2	
	13	...	...	...	...	1	2	1	3	3	1	4	2	1	1	...	...	1	...	...	...	...	...	...	22·85	22·51
	14	...	...	...	...	...	2	3	4	4	2	2	2	1	...	...	...	...	...	...	...	...	...	...	22·0	
	15	...	...	1	1	...	1	2	4	2	2	4	...	1	...	1	1	...	...	...	...	...	...	...	22·25	
4th Day ...	16	...	...	...	...	1	1	4	1	2	1	1	6	2	...	...	1	...	...	...	...	...	...	...	23·0	
	17	...	...	...	...	3	1	2	3	1	3	3	2	2	...	...	...	...	...	...	...	...	...	...	22·05	
	18	...	...	2	...	2	2	1	5	3	1	2	1	1	...	...	...	...	...	...	...	...	...	...	20·95	22·16
	19	...	...	...	1	1	3	4	4	2	1	1	1	2	...	...	...	...	...	...	...	...	...	...	21·2	
	20	...	...	...	...	...	1	...	3	2	5	...	5	3	...	1	...	...	...	...	...	...	...	...	23·6	
5th Day ...	21	...	...	1	...	2	1	3	...	...	3	5	...	2	2	...	...	1	...	...	...	...	...	...	22·8	
	22	...	...	...	...	...	...	...	3	1	...	3	2	3	5	3	...	...	...	...	...	...	...	...	25·2	
	23	...	...	...	...	...	1	...	2	2	1	2	3	1	1	2	4	...	1	...	...	...	...	...	25·35	24·72
	24	...	...	...	1	...	...	...	1	2	1	4	3	2	1	5	...	...	...	...	...	...	...	...	24·75	

6th Day ...	26	...	...	...	1	...	1	3	1	5	4	4	1	...	...	...	...	...	...	...	...	...	...	22·0	
	27	...	...	...	1	...	1	2	2	1	5	1	2	2	3	...	...	...	...	...	...	...	...	23·1	
	28	...	...	...	...	1	...	1	...	3	...	5	2	3	4	...	1	...	...	...	...	...	...	24·45	24·19
	29	...	...	...	...	...	...	...	1	...	3	3	2	3	2	2	3	1	...	...	...	...	...	25·85	
	30	...	...	...	...	...	...	...	1	...	1	5	4	4	...	2	3	...	...	...	...	...	...	25·55	
7th Day ...	31	...	...	1	1	8	3	...	3	4	...	...	...	...	...	...	...	...	...	...	...	...	...	19·25	
	32	...	...	...	2	3	3	7	1	1	2	1	...	...	...	...	...	...	...	...	...	...	...	19·9	
	33	...	...	...	...	1	...	4	4	1	1	2	2	2	1	1	1	...	...	...	...	...	...	23·25	21·71
	34	...	...	...	...	...	...	3	3	3	1	5	2	...	2	1	...	...	...	...	...	...	...	23·2	
	35	...	...	...	1	...	...	1	4	1	4	3	3	1	1	1	...	...	...	...	...	...	...	23·15	
8th Day ...	36	...	1	...	...	...	...	2	...	1	2	1	...	6	2	3	1	...	...	1	...	...	...	25·1	
	37	1	...	...	...	...	1	1	...	1	1	3	2	3	1	4	1	1	...	...	...	...	...	24·8	
	38	...	...	...	...	1	1	1	...	...	...	2	1	1	1	2	1	1	2	3	3	...	...	27·75	26·15
	39	...	...	...	...	...	...	1	1	...	3	3	1	1	...	5	2	2	...	...	1	...	...	26·2	
	40	...	...	...	...	...	...	2	2	1	...	...	1	2	1	2	2	2	4	1	...	...	...	26·9	
9th Day ...	41	...	...	...	1	...	...	...	1	2	...	1	2	5	3	3	2	...	...	...	...	...	...	...	25·45	
	42	...	...	...	...	...	1	...	1	...	2	1	4	3	3	2	...	1	2	...	...	...	...	25·85	
	43	...	...	1	...	1	...	1	...	1	...	1	3	3	4	1	2	...	1	1	...	...	...	25·5	25·67
	44	...	...	...	...	...	...	...	...	1	3	1	3	3	4	4	...	1	...	...	...	...	...	25·9	
	45	...	1	...	...	...	...	1	...	...	1	2	3	1	5	3	3	...	...	...	...	...	...	25·65	
10th Day ...	46	...	...	...	...	1	...	1	...	1	...	2	2	2	5	3	2	...	1	...	...	...	...	25·9	
	47	...	...	...	...	...	...	1	1	...	1	4	2	5	1	2	3	...	...	...	...	...	...	25·5	
	48	...	...	...	...	...	...	...	...	...	...	3	1	6	3	2	2	2	1	...	...	...	...	26·05	25·98
	49	...	...	...	...	...	1	...	2	...	2	4	2	1	1	3	1	3	...	...	...	...	...	25·45	
	50	...	...	...	1	...	...	...	...	1	3	...	2	2	5	2	2	1	...	...	1	...	...	26·1	
Totals	...	2	2	9	14	38	40	70	78	71	90	106	99	116	79	87	52	21	14	6	5	1	...	...	Average length of 1000 *T. r.* is 24·121 μ	

* These numbers refer to the consecutive groups of twenty trypanosomes, summarised on each line.

TABLE II.—Summary of measurements (in microns) of lengths of 1,000 individuals of *Trypanosoma rhodesiense* from a single white rat.

		Maximum	Minimum	Averages of each 100	Averages of each 20	Range of averages of each 20
	1	30	21		26·0	
	2	28	19		24·05	
1st Day	3	30	19	25·14	25·6	1·95
	4	29	18		24·1	
	5	31	22		25·95	
	6	29	20		25·65	
	7	29	23		26·1	
2nd Day	8	28	16	22·98	21·1	5·25
	9	30	16		20·85	
	10	26	16		21·2	
	11	27	19		23·25	
	12	27	14		22·2	
3rd Day	13	30	18	22·51	22·85	1·25
	14	26	19		22·0	
	15	29	16		22·25	
	16	29	18		23·0	
	17	26	18		22·05	
4th Day	18	26	16	22·16	20·95	2·65
	19	26	17		21·2	
	20	28	19		23·6	
	21	30	16		22·8	
	22	28	21		25·2	
5th Day	23	31	19	24·72	25·35	2·7
	24	28	17		24·75	
	25	34	20		25·5	
	26	25	17		22·0	
	27	27	17		23·1	
6th Day	28	29	18	24·19	24·45	3·85
	29	30	21		25·85	
	30	29	21		25·55	
	31	22	16		19·25	
	32	24	17		19·9	
7th day	33	29	18	21·71	23·05	3·95
	34	28	20		23·2	
	35	28	17		23·15	
	36	32	15		25·1	
	37	30	14		24·8	
8th Day	38	33	18	26·15	27·75	2·95
	39	33	20		26·2	
	40	32	20		26·9	
	41	29	17		25·45	
	42	31	19		25·85	
9th Day	43	32	16	25·67	25·5	0·45
	44	30	22		25·9	
	45	29	15		25·65	
	46	31	18		25·9	
	47	29	20		25·5	
10th Day	48	31	24	25·98	26·95	1·45
	49	30	19		25·45	
	50	33	17		26·1	
					Range = 27·75—19·25 = 8·5	

TABLE III.—*T. rhodesiense*, in which the trypanosomes are arranged in Bruce's three groups (*a*) 13-21μ; (*b*) 22-24μ; (*c*) 25μ and upwards.

Day	1	2	3	4	5	6	7	8	9	10	Totals
Stumpy ... 13–21μ	12	41	37	45	17	16	53	15	9	8	253
Intermediate 22–24μ	25	19	40	28	31	40	29	18	16	21	267
Long ... 25–36μ	63	40	23	27	52	44	18	67	75	71	480
	100	100	100	100	100	100	100	100	100	100	1,000

This table shows very clearly what we have already pointed out, namely, the great variation in the figures for each group on particular days. Thus on the tenth day there were 8 % of stumpy forms, while on the seventh day there were 53 %. This seems to us to make it perfectly obvious that when a sample is taken at random from an animal on any day an erratic factor is introduced.

We proceed to represent the preceding results graphically.

Chart I shows a curve of measurement of *Trypanosoma rhodesiense*. The distribution, by percentages in respect to length, of the 1,000 non-dividing specimens of the trypanosome is plotted. The parasites were taken from the peripheral blood of a rat. One hundred trypanosomes were measured each day for ten consecutive days of infection (vide Table I).

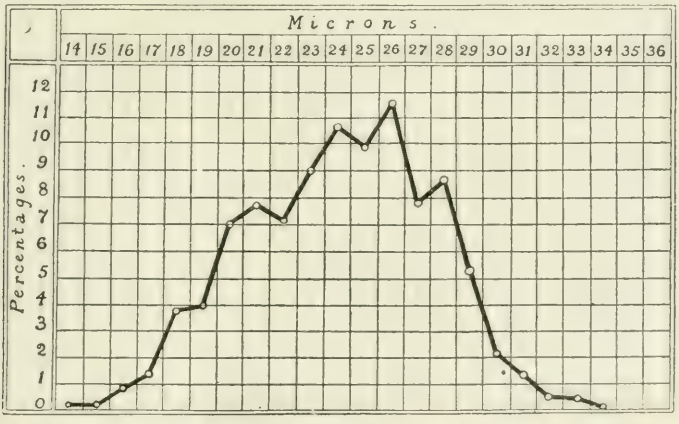

CHART I.—Graphical representation of the distribution of the lengths of 1,000 *T. rhodesiense* from one rat.

We next proceed to give corresponding tables for *T. gambiense*.

TABLE IV.—Distribution in respect to length of 1,000 non-dividing individuals of *Trypanosoma gambiense* from a single white rat.

		In Microns																						Averages	
		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	of each 20	of each 100
1st Day ...	1	...	...	...	1	1	1	2	1	5	...	5	1	2	1	...	...	...	...	...	...	...	...	...	22·45	
	2	...	...	...	...	...	...	...	1	3	3	5	3	2	1	...	...	1	...	1	...	...	...	...	24·6	
	3	...	...	...	...	1	1	3	3	6	...	2	2	...	1	1	...	...	...	...	...	...	...	...	22·25	24·16
	4	...	...	...	...	...	...	...	...	2	...	3	4	2	3	1	5	...	...	...	...	...	...	...	26·1	
	5	...	...	...	...	...	...	...	2	2	2	2	1	3	3	1	4	...	...	...	...	...	...	...	25·4	
2nd Day ...	6	...	...	...	...	...	...	...	...	2	3	2	1	1	1	3	3	1	2	1	...	...	...	...	26·7	
	7	...	...	...	1	...	...	...	...	...	...	1	3	6	2	4	2	...	1	...	...	...	...	...	26·35	
	8	...	...	...	...	...	...	...	...	...	...	3	...	5	4	4	3	1	...	...	...	...	...	...	26·95	26·65
	9	...	...	...	...	...	...	...	...	1	1	...	8	...	4	4	1	1	...	...	...	...	...	...	26·2	
	10	...	...	...	...	...	...	...	...	...	2	1	1	3	4	2	6	1	...	...	...	...	...	...	27·05	
3rd Day ...	11	...	...	...	...	1	...	...	3	1	2	2	5	2	2	...	2	...	...	...	...	...	...	...	24·3	
	12	...	...	...	1	...	...	2	1	1	...	4	4	3	1	...	1	...	1	1	...	...	...	...	24·65	
	13	...	...	1	1	...	1	2	5	2	2	3	...	1	1	1	...	...	...	...	...	...	...	...	22·0	24·14
	14	...	...	...	1	...	...	1	3	3	4	2	1	...	3	2	...	...	...	...	...	...	...	...	23·4	
	15	...	...	...	...	...	...	...	2	...	2	1	3	1	3	1	5	2	...	...	...	...	...	...	26·35	
4th Day ...	16	...	...	...	...	...	...	...	2	2	1	3	2	1	1	2	1	...	1	1	1	1	...	1	26·75	
	17	...	...	...	...	...	...	...	1	2	...	1	...	1	4	3	5	...	3	...	...	...	...	...	27·25	
	18	...	...	...	...	...	1	...	1	...	3	3	2	2	3	3	1	1	...	...	...	...	...	...	25·35	26·42
	19	...	...	...	...	...	...	...	2	1	1	1	1	...	2	5	2	1	3	1	...	...	...	...	27·15	
	20	...	...	...	...	...	...	...	3	2	...	3	4	1	...	1	3	2	...	1	...	...	...	...	25·6	
5th Day ...	21	...	...	...	...	...	2	3	3	1	7	2	1	1	...	...	...	...	...	...	...	...	...	...	22·15	
	22	...	...	1	...	...	...	...	3	1	1	2	4	4	1	2	1	...	...	...	...	...	...	...	24·4	
	23	...	...	...	...	...	...	1	...	2	2	4	3	4	3	...	1	...	...	...	...	...	...	...	24·75	23·87
	24	...	...	...	...	...	1	...	2	2	2	4	2	2	2	1	1	...	1	...	...	...	...	...	24·55	
	25	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	

6th Day ...	26	...	...	...	...	...	...	2	...	1	6	3	1	5	...	...	1	...	...	1	...	...	...	...	...	...	
	27	...	...	...	1	...	...	2	2	3	4	3	2	3	...	...	...	...	...	...	...	...	...	...	...	22·85	
	28	...	...	...	...	...	1	1	1	4	6	2	1	1	2	1	...	...	...	...	...	...	...	...	...	23·35	22·88
	29	...	...	...	1	2	...	2	4	3	5	...	...	...	2	1	...	...	...	...	...	...	...	...	...	22·0	
	30	...	...	...	...	...	2	5	3	1	4	4	1	...	...	...	...	...	...	...	...	...	...	...	...	21·8	
7th Day ...	31	...	...	1	...	1	1	4	1	3	5	2	2	...	...	...	...	...	...	...	...	...	...	...	...	21·65	
	32	...	...	...	...	1	4	1	4	3	5	2	...	...	...	...	...	...	...	...	...	...	...	...	...	21·35	
	33	...	...	...	2	1	4	3	2	3	...	...	3	2	...	...	...	...	...	...	...	...	...	...	...	21·15	21·07
	34	...	...	1	...	1	2	7	3	1	3	...	...	1	1	...	...	...	...	...	...	...	...	...	...	20·95	
	35	...	...	1	2	3	3	2	2	3	3	...	...	...	1	...	...	...	...	...	...	...	...	...	...	20·25	
8th Day ...	36	...	...	...	...	...	...	1	1	...	...	1	3	6	3	3	1	1	...	...	...	...	...	...	...	26·0	
	37	...	...	...	...	...	...	1	...	2	...	1	3	5	5	1	1	...	1	...	...	...	...	...	...	25·8	
	38	...	...	...	...	...	...	...	...	2	...	2	3	2	1	2	3	4	1	...	...	...	...	...	...	27·0	26·37
	39	...	...	...	...	...	...	...	1	1	...	1	2	1	1	2	7	1	2	1	...	...	...	...	...	27·65	
	40	...	...	...	...	...	...	1	1	1	4	...	2	2	5	...	4	...	...	...	...	...	...	...	...	25·4	
9th Day ...	41	...	...	...	...	...	...	...	1	1	1	2	5	3	1	4	2	...	...	...	...	...	...	...	...	25·7	
	42	...	...	...	...	...	1	...	...	3	...	2	1	2	2	3	3	3	...	...	...	...	...	...	...	26·25	
	43	...	...	...	...	...	...	...	1	...	2	4	2	5	3	1	1	...	...	1	...	...	...	...	...	25·65	26·17
	44	...	...	...	...	1	...	...	1	2	...	...	4	1	3	4	3	1	...	...	...	...	...	...	...	25·95	
	45	...	...	...	...	...	...	...	...	1	...	2	1	3	3	5	2	1	1	...	1	...	...	...	...	27·3	
10th Day ...	46	...	...	...	...	...	1	...	...	1	...	...	2	3	1	2	4	5	...	1	...	...	...	...	...	27·5	
	47	...	...	...	...	...	...	...	1	1	...	...	...	3	1	6	6	1	...	...	...	1	...	...	...	27·7	
	48	...	...	...	1	...	1	1	...	...	...	4	...	1	2	1	3	3	2	1	...	...	...	...	...	26·55	26·94
	49	...	...	...	1	...	...	1	1	...	2	1	3	1	3	1	1	3	1	1	...	...	...	...	...	26·90	
	50	...	...	...	...	...	...	...	1	1	1	1	1	5	2	1	2	3	1	...	1	...	...	...	...	26·05	
Totals	...	...	...	5	13	16	28	49	71	84	89	97	100	105	95	80	91	38	21	12	3	2	0	1		Average length of 1000 T.g. is 24·867 μ	

TABLE V.—Summary of measurements (in microns) of lengths of 1,000 individuals of *T. gambiense* from a single white rat

		Maximum	Minimum	Averages of each 100	Averages of each 20	Range of averages of each 20
	1	27	17		22·45	
	2	32	21		24·6	
1st Day	3	28	18	24·16	22·25	3·85
	4	29	22		26·1	
	5	29	21		25·4	
	6	32	22		26·7	
	7	31	17		26·35	
2nd Day	8	30	24	26·65	26·95	0·85
	9	30	22		26·2	
	10	30	23		27·05	
	11	29	18		24·3	
	12	32	17		24·65	
3rd Day	13	28	16	24·14	22·0	4·35
	14	28	17		23·4	
	15	30	21		26·35	
	16	36	21		26·75	
	17	31	21		27·25	
4th Day	18	30	19	26·42	25·35	1·9
	19	32	21		27·15	
	20	32	21		25·6	
	21	26	19		22·15	
	22	29	16		24·4	
5th Day	23	29	20	23·87	24·75	2·6
	24	31	19		24·55	
	25	30	18		23·5	
	26	32	20		24·4	
	27	26	17		22·85	
6th Day	28	28	19	22·88	23·35	2·6
	29	28	17		22·0	
	30	25	19		21·8	
	31	25	16		21·65	
	32	24	18		21·35	
7th Day	33	26	17	21·07	21·15	1·4
	34	27	16		20·95	
	35	27	16		20·25	
	36	30	20		26·0	
	37	31	20		25·8	
8th Day	38	31	22	26·37	27·0	2·25
	39	32	21		27·65	
	40	29	20		25·4	
	41	29	21		25·7	
	42	30	19		26·25	
9th Day	43	32	21	26·17	25·65	1·65
	44	30	18		25·95	
	45	33	22		27·3	
	46	32	19		27·5	
	47	34	21		27·7	
10th Day	48	32	17	26·94	26·55	1·7
	49	32	17		26·00	
	50	33	21		26·95	
					Range = 27·7—20·25 = 7·45	

TABLE VI.—*T. gambiense*, in which the trypanosomes are arranged in Bruce's three groups.

Day	1	2	3	4	5	6	7	8	9	10	Totals
Stumpy ... 13–21μ	17	1	25	10	23	29	57	6	5	9	182
Intermediate 22–24μ	40	16	29	23	33	49	33	15	20	12	270
Long ... 25–36μ	43	83	46	67	44	22	10	79	75	79	548
	100	100	100	100	100	100	100	100	100	100	1,000

Here again we note a great variation in the figures for each group, for example, on the second day 1 % of stumpy forms, and on the seventh day 57 %. The differences are due, according to some authors, to a cycle in the vertebrate host.

In Chart II we give a curve representing the distribution, by percentages in respect to length, of the 1,000 non-dividing specimens of *Trypanosoma gambiense*, taken from a rat. One hundred trypanosomes were measured each day for ten consecutive days of infection (*vide* Table IV).

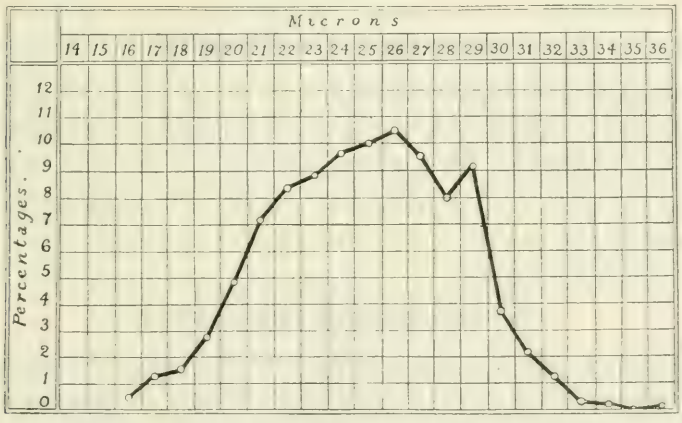

CHART II.—Graphical representation of the distribution of the lengths of 1,000 *T. gambiense* from one rat.

Comparing now *T. gambiense* and *T. rhodesiense* we get the following tables.

TABLE VII

	Average length	Maximum	Minimum
T. gambiense	24·87μ	36·0μ	16·0μ
T. rhodesiense	24·12μ	34·0μ	14·0μ

TABLE VIII.—Comparison of distribution of the trypanosomes according to Bruce's groups.

	13—21μ	22—24μ	25μ and upwards
T. gambiense	18·2 %	27·0 %	54·8 %
T. rhodesiense	25·3 %	26·7 %	48·0 %

TABLE IX.—Distribution by Octiles of both *T. gambiense* and *T. rhodesiense.*

	125th	250th	375th	500th	625th	750th	875th
T. gambiense	21μ	22μ	24μ	25μ	26μ	27μ	29μ
T. rhodesiense	20μ	21μ	23μ	24μ	26μ	27μ	28μ

DISCUSSION OF RESULTS

The fact that we are dealing with a dimorphic trypanosome, the dimorphic nature of which is not thoroughly understood, is no doubt responsible for the difference of opinion as to procedure and for the different results obtained. Such difficulties probably do not arise in the case of a monomorphic trypanosome.

I. THE SIZE OF THE SAMPLE

If Tables II and V be examined, it will be found that on one day the average values of five samples of twenty trypanosomes may vary by as much as 4·35μ in the case of *T. gambiense* and by 5·25μ in the case of *T. rhodesiense.* Also the differences between

the average value for a sample of 100 on any particular day and any of the samples of 20 for that day may vary by as much as 2·21μ (third day) in the case of *T. gambiense*, and 3·12μ (second day) in the case of *T. rhodesiense*, but in the majority of cases it is only about 1 μ. From this it may be inferred that a sample of 100 is a fairly reliable one when trypanosomes are plentiful in the blood; it will, however, not be so good a sample as when trypanosomes are scanty.

II. THE INFLUENCE OF THE DAY OF INFECTION

As was indicated clearly in our previous paper, the day of infection in an acute trypanosomiasis (when death ensues in about ten days), such as that with which we have been dealing, is very important in determining whether the trypanosomes are short or long. Thus on examining our results (arranged according to Bruce's groups) in Table VI, we find that stumpy forms vary from 1 % on the second day to 57% on the seventh day, and this variation is, of course, reflected in the average values of 100 for those days, viz., 26·65μ and 21·07μ respectively. Whether these differences occur in so marked a degree in a chronic infection we are not in a position to state.

III. THE INFLUENCE OF THE ANIMAL HOST

As the day is of prime importance, it is impossible to say whether the length of any trypanosome varies markedly in different hosts. So far as we can see, this could only be determined by measuring 1,000 trypanosomes (if this number suffice) from each of the hosts in question. Consequently we consider it is advisable at present to measure from the same species of animal if comparisons are to be of value.

IV. COMPARISON OF OUR RESULTS WITH THOSE OF OTHER OBSERVERS

Curves for *T. rhodesiense* have been constructed by Bruce and his collaborators (1912), by Kinghorn and Yorke (1912), and by ourselves. There is a fair correspondence between those of Bruce and ourselves, but none between those of Kinghorn and Yorke and

ourselves. We cannot, unfortunately, in the present state of our knowledge, explain these differences satisfactorily. We believe that they must be due to difference of method, namely, that other observers have taken different species of animals on a variety of days.

We ourselves have now completed three curves (1) based on 1,000 trypanosomes from various animals, but including 600 from rats; (2) based on 600 trypanosomes from rats alone, where samples of twenty were taken on a variety of days from several rats; and now finally (3) 1,000 trypanosomes comprising 100 a day from one rat for ten days. These three curves have this in common, that each one has its main peak at 26μ. Further, the agreement is most close between curves (2) and (3) based on rats alone.

We have given previously our reasons for believing that the third method is the best, and the agreement that exists between our curves indicates, we believe, the consistency of our method. We must admit that Kinghorn and Yorke's three curves are also consistent, but it is noticeable that in their measurements, though different animals were used, the total number of trypanosomes from each animal was the same in each case. There is further the possibility that trypanosomes direct from the natural vertebrate hosts or the fly have a different character from those that have been maintained in laboratory animals.

CONCLUSION

We must admit that we had hoped to be able to distinguish between the two species, *T. rhodesiense* and *T. gambiense*, by measuring one thousand specimens of each organism. Though these biometric results are not sufficiently conclusive, we think that it is generally admitted that the two species are distinct.

Microscopically, the two trypanosomes are indistinguishable except by the posterior nuclear character of *T. rhodesiense*. We believe that a curve only expresses graphically what the eye can appreciate under the microscope, and that if two trypanosomes cannot be distinguished microscopically, we shall not be able to do so by measuring them. However, provided that further experience enables observers to agree as to the best procedure, it is no doubt

a great advantage to have a correct graphical expression for what is otherwise only an impression, although it may be a quite accurate one. Further, these measurements should not be regarded as useless, as they will undoubtedly form the basis (provided all the protocols are given) for a critical statistical investigation in the future.

We have pleasure in thanking Mr. Walter Stott, Honorary Statistician to the Liverpool School of Tropical Medicine, for kindly examining our figures.

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A NEW MOSQUITO-PROOF AND STORM-PROOF HOUSE FOR THE TROPICS

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WEST AFRICAN MEDICAL STAFF

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PLATE IV

There are all kinds of houses in the tropics. There is the old house built on enormous piles so that its occupants may be well away from the damp of the ground; there is the house built on the ground; and there is the house that is raised on short pillars only three or four feet high.

Provision for ventilation is equally varied. In olden days the houses were designed in England, and the window area had the same relation to the floor area as obtains here; then the window area was increased to one-tenth that of the floor area; this proportion was again gradually increased, until now in some places a window area equal to one-half of the floor area is regarded as the minimum necessary for comfort.

The discovery of the transmission of malarial fever by mosquitos was followed by the grafting on of mosquito-proof wire gauze to the windows and doors of existing houses, with the result that in those in which the ventilation space was small, the rooms were made insufferably hot and stuffy; consequently, the mosquito-proofed window was made to open and shut and the house was turned into a mosquito trap.

With the closing in of the house by mosquito-proofing came the growth of the verandah and the diminution in size of the rooms, the verandah now being used as a living room except during cold or stormy weather.

From these few remarks it is clear that, even taking into account the demands of local conditions, house construction in many parts of the tropics is not based on such fixed principles to guide architects as obtain, for instance, in England.

The importance of efficient mosquito protection is well known. Its value as compared with quinine prophylaxis against malaria may be gauged from the following figures of Celli:—

Treatment	Infected with Malaria
Mosquito protection plus quinine	1·75 %
Mosquito protection alone	2·5 %
Quinine alone	20·0 %
No protection	33·0 %

The difficulty of completely protecting a house raised on pillars from mosquitos may also be noted. Faults in the flooring nearly always exist which allow of the entry of mosquitos.

Dr. Leonard Hill states that to render a room uncomfortably hot and stuffy, the air in it must be hot, damp and still. In many parts of the tropics the first two of these conditions are almost constant, the last only therefore concerns us here.

Three factors tend to keep still the air enclosed in a mosquito-proofed house.

1. The mosquito proofing itself, by shutting off more than 30 % of the space to which it is applied.

2. The loss of air movement owing to friction with the wires and to the interference with the air particles which impinge on the netting.

3. The division of the house by walls into rooms and passages hindering through ventilation.

Factors 1 and 2 should strictly be considered together, as a certain amount of the air impinging on the wires will ultimately pass through the netting. But without attempting the general consideration of the motion of the air, it is clear that for a given uncovered area the loss of movement will be greater for a small mesh than for a large one, on account of the greater length of edge of wire. If the uncovered area be equal to an open square of side a and consist of n small squares each way, then, as each side of a wire has its effect on the air movement, the total length of edge is $n^2 \times 4\left(\frac{a}{n}\right) = 4an$.

Messrs. Humphreys, Ltd., of Knightsbridge—the makers of the first mosquito-proofed house used in Sir Patrick Manson's classical experiment—have designed for me a house for use in the tropics embodying certain principles which I believe are new.

The house here illustrated (Plate IV) is constructed almost entirely of steel; wood-work is reduced to a minimum, and, where its use is unavoidable, is specially treated to withstand the attacks of insects, especially white ants.

The house is built on a plinth of concrete and has a floor and a low wall of similar material. Their surfaces are cement-rendered. The walls are continuous with a steel framing, filled in completely with mosquito netting made of a specially woven composite material proved to be stronger and more durable than any other. This netting is sandwiched between perforated metal sheets, which prevent bulging and render entry impossible except by the spring doors at each end of the lobby-entrances.

The roof is covered with non-conducting fibro-cement slates and is ventilated by means of louvre windows at each end. It is completely shut off from the room below by an asbestos ceiling.

There are no walls or partitions within the mosquito proofing, so that whatever breeze there may be, blows straight through the house from one side to the other. Should this wind be too cold or too strong, as for instance during storms, it can be cut off at will, by shutters which are provided all round the house and which are capable of closing the whole of it. These are manipulated from within by turning a handle, and can be made either to come down from above, as illustrated, or to move upwards from below.

The division of the interior of the house is left to the occupant, the ideal being the minimum required for privacy, and it is suggested that by the use of sun blinds, screens and curtains, the open character of the house may be preserved, but permanent brick or stone walls may be built if desired.

It is possible to make the house of any number of stories, and to build it on piles if desired.

In the house we have been considering that the terms *wall area* and *available ventilation* space are almost synonymous; it must not be forgotten, however, that the proportion between wall space and cubic content vary greatly according to the size and shape of the building.

In buildings of the same shape the cubic content varies as the square of the wall area, provided that the height is constant; while a square house has a greater cubic content for a given wall area than an oblong one.

In conclusion I have to thank Sir Ronald Ross and Professor Simpson for their interest, and for many useful criticisms and suggestions. I have also to thank Messrs. Humphreys, Ltd., not only for the amount of work they have done, but also for their courtesy.

CONTRIBUTIONS TO THE STUDY OF COLOUR MARKING AND OTHER VARIABLE CHARACTERS OF *ANOPHELINAE* WITH SPECIAL REFERENCE TO THE SYSTEMATIC AND PHYLOGENETIC GROUPING OF SPECIES

BY

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PLATES V-VIII

CONTENTS

PAGE

I. VARIATION IN COLOUR MARKING OF *Anophelinae* 45
II. ORNAMENTATION OF THE WINGS IN *Anophelinae* 52
Nodal Leucogenetic Centres of the Wing ... ... 5[illegible]
Internodal Leucogenetic Centres of the Wing 61
Variation in Colour Marking shown by Individuals of the same Species... 65
III. VARIATION IN STRUCTURE OTHER THAN THAT CONNECTED WITH SCALES 65
IV. STRUCTURAL VARIATION IN THE IMMATURE STAGES 72
V. VARIATION AS DISPLAYED BY SCALES 75
VI. CLASSIFICATION, PHYLOGENY AND GEOGRAPHICAL DISTRIBUTION OF THE *Anophelinae* 81
VII. GENERAL CONCLUSIONS 85
VIII. TABULAR SUMMARY 87
REFERENCES 93
EXPLANATION OF PLATES 94

I. VARIATION IN COLOUR MARKING OF *ANOPHELINAE*

Nature of colour marking. With few exceptions, the markings of Anophelines are due to the varied distribution of light and dark scales. A mixture of light and dark scales may occur, e.g., on the wing veins of certain species or on the legs when these show mottling; but the most striking feature of the ornamentation of the sub-family is the occurrence of patches of either dark or light scales forming dark or light spots respectively. Where the dark scales predominate the appearance is that of light spots on a dark surface; where, on the other hand, the extent of light scaling is greater, an appearance of dark spots on a light ground is produced.

Leucogenetic centres and residual pigment areas. In a great many species the *scheme* of coloration is similar, the actual markings being brought about merely by different degrees of development of the scheme. The process of advancing ornamentation is one of increased whitening, and consists in the progressive appearance and spread of pale areas which have their origin at certain fixed points (leucogenetic centres).

Although the pale areas developing from these centres may spread until they coalesce with neighbouring pale areas, they do not, as a rule, do this, and there is a distinct hesitation shown at the final extinction of intervening dark areas. Also extension of the pale areas does not necessarily encroach equally on both sides of a dark area, and the behaviour of markings in this respect suggests that there exist something very like actual pigment centres having a definitely fixed position. Points at which the pigment seems thus to make a last stand will be referred to as *residual pigment areas*, or simply as pigment centres. In some cases centres become quite obliterated, some being apparently less resistant than others, but even in a high degree of whitening, such as frequently occurs in the case of the wing, where many of the pigment areas are displayed as mere points, the number actually present is not much reduced. A row of these pigment centres lying near the ends of the longitudinal veins of the wing constitute the marginal spots of Donitz, and others have an equally fixed and characteristic nature.

By referring markings to the leucogenetic centres which produce them (and so far as one can see, these centres of whitening are a very real phenomenon), and to the position of the pigment centres, the study of colour markings is greatly simplified.

Position of colour markings. The most important colour markings are those of the palps, legs and wings. There are, of course, variations in the coloration of other parts of the body which may assume importance in particular species.

Almost all Anophelines have a head covered with dark scales behind, and with a ' forehead ' patch of pale white or creamy scales prolonged forward as a tuft between the eyes. The *absence* of this marking would be an important feature in any species.

In the case of the proboscis, only a few species show any ornamentation other than the light tip due to the fact that the

labellae are not dark scaled like the rest of the proboscis. But a very striking variation is seen in a few species where the outer portion of the proboscis is pale or snowy white.

Markings of the palps. A certain number of species show palps without any markings (unbanded palps). In some cases there is a faint indication of bands, due very often to the chitin showing at the joints, but there is no actual pale ornamentation. This is the condition which is sometimes referred to as palpal banding in *A. nigripes*, Staeger. *A. immaculatus*, Theo., also stated to have banded palps, shows very indefinite and indistinct ones, not at all sharply marked as in ordinary Anophelines.

Entirely unornamented palps are present in all *Stethomyias*, including *S. corethroides*, Theo. (vide section on scale structure), and *S. aitkeni*, James and Liston. Unbanded palps are also commonly present in *Anopheles*, and occur in *Myzorhynchus* and *Cycloleppteron*. Otherwise the palps are usually ornamented.

In *Anopheles* the unspotted wing species also have unbanded palps (except *A.* (?) *smithi*, Theo.*). The species *A. gigas*, Giles, and *A. simlensis*, James, with banded palps have been separated by James[1] as *Patagiamyia*. *A. formosus*, Ludlow, since it is a hill species whose description closely resembles that of *gigas*, would seem also to be a *Patagiamyia*. *A. punctipennis*, Say., which is a *Patagiamyia* (vide section on scale structure), has banded palps. It would be very interesting to know what was the condition of the prothoracic lobes in *A. eiseni*, *A. crucians*, etc. *A. welcomei*, Theo., is a peculiar species; it has no prothoracic tuft, but I am doubtful if it should be considered as an *Anopheles*.

When the palps are ornamented, this is in the form of bands, supplemented in some cases by extra spots of white (speckling) on certain segments.

It is usual to talk of four or three-banded palps, etc., some of the bands being described as broad or narrow, as the case may be. The only accurate method is to refer to the position of the bands in respect to the palpal segments. This relation is most easily expressed in terms of leucogenetic centres. In spite of considerable variety of appearance, a comparatively few general schemes of coloration associated with different relative lengths of the palpal

* Note.—This species has a scale tuft on the prothorax and is therefore not an Anopheles in the sense of James.

segments (vide section on structural variation) can be made to account for the markings.

SCHEME A. Palps with leucogenetic centres at each end of the segments, but none of these actively spreading.

The effect of the appearance of the leucogenetic areas (following upon complete absence of banding) in this scheme is the production of four narrow pale bands, including the pale apex. If the species be orthodactylous†, a somewhat different appearance is given to that produced in the case of a markedly heterodactylous* species (compare *C. pulcherrima* with the variety of *P. nursei* showing a dark apical band. Plate V, figs. 8 and 10).

This degree of extension of whitening is not exceeded in *Myzorhynchus*, and is typical of palpal ornamentation seen in *Myzorhynchus*, *Cycloleppteron*, *Arribalzagia*, *Patagiamyia* and *Cellia*.

It seems to be a stable condition, and is but little liable to variation in individuals belonging to these groups. In *Myzomyia*, *Pyretophorus* and *Nyssorhynchus* this stage is unusual, and when it does occur it is most often as an individual variation, e.g., the four-banded palp varieties of *N. fuliginosus*, *Ne. fowleri* and *P. nursei*. A permanent four-banded condition in this group is seen in *P. cinereus*, Theo.

SCHEME B. Leucogenetic centres at the ends of the segments, with that at the end of the apical segment active.

In this scheme the apical centre extends, making the apical segment entirely pale. This results in the ordinary type of three-banded palp. Considerable difference in general appearance again depends on whether the palp is orthodactylous or heterodactylous (Plate V, figs. 11-13).

The narrow apical band in *Myzomyia*† (*culicifacies*, *funestus*, etc.) is homologous in regard to extent of palp involved with the broad apical band of *Pm. rossi*, but not with the narrow apical band in *Pt. similensis* or *C. pulcherrima*. The broad band in

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* *Vide* Section III.

† NOTE.—As pointed out by Edwards[6], *Nyssorhynchus*, Blanchard (= *Laverania*, Theo.) having as type species *argyrotarsis*, Rob-Desv., must take precedence over *Cellia*, Theo., a new name being required for the group generally known as *Nyssorhynchus*. Again *rossi*, Giles, is the type species of *Myzomyia*, Blanchard (= *Grassia*, Theo.) so that the name *Pseudomyzomyia*, Theo., is incorrect, whilst a new name is required for what is now generally known as *Myzomyia*. In the present paper, however, the names in general use by Theobald are retained as being less confusing than new ones or old ones applied in a new sense.

Pm. rossi again is not comparable with the broad apical band in *N. maculatus*. (Plate V, fig. 14.)

Certain species, as previously mentioned, which normally or sometimes exhibit this condition (i.e., whitening of the whole apical segment) may at other times show a black band, due to incomplete extension of the apical pale area. The liability to this variation is not merely accidental. In the case of *Nyssorhynchus*, those species liable to the variation are distinctly related and exhibit affinities to *Cellia*. In *Myzomyia* incomplete extension of the apical white area is rare. It is more common in the heterodactylous *Pyretophorus*, e.g., *P. cinereus*, as a variation in *P. nursei*, etc.

A few species, all apparently related, show a peculiar variant of palpal banding, due to the absence of development of the apical pale area. These dark-tipped species which show three bands are *M. turkhudi*, Liston, *M. hispaniola*, Theo., *P. myzomifacies*, Theo., and *P. chaudoyei*, Theo.

In *M. turkhudi* this character is liable to some variation, the dark tip not being always very clearly shown.

SCHEME C. So far, the only leucogenetic centre that has shown active spreading properties is that at the apex of the terminal segment. Another type of palp is seen in which the centres at both ends of the penultimate segment and at the apex of the second segment also become active. In this case whitening spreads up and down the shafts of the segments to produce the appearance seen in *N. maculatus* (Plate V, fig. 14). Here the residual pigment area is displayed as occupying almost the exact middle of the segment. The apical white band in such a case is not comparable with what looks like a very similar band in the case of *Pm. rossi*, etc.

Variation here usually consists in a partial, or more rarely in a complete effacement of the pigment centre on the penultimate segment.

SCHEME D. What seems to be essentially a distinct scheme of ornamentation to any of the above, is one which may be described as showing in each segment only one apically situated leucogenetic centre and a basally situated residual pigment area. Species showing this type of palpal ornamentation are chiefly included under the many-spotted-winged species of Donitz, i.e., *M. punctulata*, *Nm. elegans*, *N. annulipes*, etc.

The scheme is beautifully demonstrated in *Ch. kochi*, Donitz, and it is apparently that on which the palpal ornamentation of *N. karwari*, James and Liston, is based (vide Plate V, fig. 15). The condition is very distinct from that ordinarily seen in *Nyssorhynchus*.

Possibly other variations or schemes of palpal ornamentation exist, but enough has been said to show the nature of the condition present in the great majority of species.

Markings of the legs. The occurrence of pale areas at the terminations of the femur, tibia and first tarsus is usual, and such may be conveniently termed '*knee spots*.' Except as an unusual variation, knee spots appear to be absent only in *Stethomyia*.

The occurrence of pale areas at the termination of the tarsal segments gives rise to an appearance which may be called *tarsal banding*. Tarsal banding may be of several kinds, depending upon whether the banding is a mere trace not very definite and confined to the actual joint, or whether it is broad and distinct involving an appreciable extent of the ends of the segments. In some cases, though banding is narrow, it is (as in *P. jeyporensis*) very distinct. In the case of broad banding, there are again two conditions depending upon whether the light area is merely somewhat paler than the rest of the segment or is pure white. The latter condition is, of course, much more conspicuous.

In some species the tips of the hind legs (and very rarely those of other legs) are white. This may be a condition in which there is a large continuous white area (as in *N. fuliginosus*, *My. paludis*, etc.), or one in which the whole tarsus shows alternate dark and light bands, one of which includes the tip. In the former case it will be found that two or three segments are completely devoid of any dark scales. In the latter case, though the terminal segment may be all white, the other segments still have some portion of their extent dark. In some cases, though the tip is white, not even one segment is altogether white, the terminal portion of white consisting in this case, as a rule, of a half or less, of the last tarsal segment. In separation of species the exact details regarding the number of segments white is important, but in the present paper, dealing somewhat broadly with the subject, I shall refer to two conditions only, i.e., (*a*) a continuous area of white involving at

least two segments; (*b*) an interrupted black and white appearance of the tarsus in which the tip is white (i.e., as in *N. maculatus*, Theo.).

In the case of the tarsus of certain Culicinae there may be a development of pale areas at both ends of the segment or at one or other end only, so that there results apical and basal, apical, or basal banding as the case may be. Seeing how important the coloration scheme appears to be, such distinctions may have a good deal more systematic importance than one might be ready to grant at first sight. So far I have not sufficiently studied the nature of broad tarsal banding, and for the present shall not lay any stress on differences in this respect. In many species, however, the banding is purely apical, whilst in *Pm. rossi*, for example, it is distinctly apical and basal.

The condition called speckling is one in which distinct spots or bands are produced on the tibia or femur. Under an objective a certain amount of admixture of dark and light scales is often seen, and this may lead to a 'mottled' appearance under a lens. This is quite distinct from the defined spots and bands referred to as speckling. Speckling signifies a high degree of ornamentation and is usually associated with tarsal banding and, very frequently, with a white tipped tarsus.

An absence of speckling is characteristic of *Myzomyia* and of the *superpictus* group of *Pyretophorus*. Speckled species of *Pyretophorus* seem to be in many ways distinct from the latter group. Note, for example, *P. ardensis*, Theo., with 'furry' palps resembling those of a *Nyssorhynchus*, and the highly ornamented *P. aureosquamiger*, Theo. *Vide* also remarks on the scale structure and palpal characters of *P. costalis*.

In *Nyssorhynchus* speckling is the rule, and reaches a high degree of perfection. In *Anopheles*, *Myzorhynchus* and *Patagiamyia*, speckling is absent, but it is developed in *Cycloleppteron*, evidently a specialised but closely related genus to *Myzorhynchus*.

Ornamentation of the wing in Anopheles. The study of wing markings is complicated by the number of veins which may bear spots, the fact that the arrangement of spots on each vein may vary and that combinations are possible.

II. ORNAMENTATION OF THE WING IN *ANOPHELINAE*

As is well known, only the veins in mosquitos carry scales, and any spots that may be present are, therefore, restricted to these structures, and are linear in shape.

In this respect the spots on Anopheles differ from those in butterflies, not only in being linear, but in the fact that the spots in butterflies, as has been shown by Mayer,[2] originate on the wing between the veins, and only involve the veins by extension.

In the Nematocera two types of spots are seen (*a*) dark spots situated around vein junctions, and (*b*) dark wavy bands with serpiginous edges which extend across the wing. These often look as if their wavy margins had been caused by encroachment of pale areas developed between the veins.

The former type of spots are constantly encountered. On page 92, for example, of Theobald's Monograph, Vol. I, is figured a wing of *Rhyphus* in which dark patches are seen (1) at the junction of the subcosta with the costa, (2) at the junction of the first and upper branch of the second with the wing edge, (3) at the termination of the lower branch of the second, (4) at the cross vein linking the second with the next vein, and at two other junctions.

The most striking development of pale spots forming between the veins, and dark spots in connection with vein junctions I have seen has been in the case of certain species of spotted-winged *Chironomus*. In these species both wing membrane and vein is covered with minute hairs not unlike scales, and so the condition approaches that seen in Anophelines, whilst it seems to show what the primitive condition may have been. In these wings the two conditions seen are (1) aggregations of the hairs (or scales) in dense patches in the neighbourhood of the main cross veins and at two areas which seem to represent the bifurcation of the second and fourth longitudinal veins, and (2) distinct pale eye-like spots which occur on the wing membrane and have the minute scales covering them also pale (Plate VI, fig. 4). In one species examined there was a complete series of such spots occupying the cells and ranging along the whole outer and posterior aspect of the wing.

I have described these conditions at some length because

I believe they help one to understand certain of the facts to be described in the case of Anophelines, and because they seem to show so distinctly the primitive character of scale aggregation at the cross veins, and the actual existence of something very like my leucogenetic centres.

Primitive spotting in Anophelinae. The spotting seen on the wings of Anopheles is of two kinds:

(*a*) Spots due to special aggregations of scales;

(*b*) Spots due to alternate areas of light and dark scales.

The first type of spotting is very familiar in the case of the wings of *A. maculipennis*, Meigen. In this species no pale scales are present on the wing veins, but there are aggregations of scales forming dark spots. Such aggregations occur at the origin of the second longitudinal, at the origin of the third longitudinal, to a less extent on the neighbouring parts of the fourth and fifth veins (i.e., in the neighbourhood of the cross veins), and at the bifurcation of the second and fourth veins. This type of spotting is conspicuous in *A. maculipennis*, because the wings are devoid of other markings; but it is by no means confined to this species or to species with unspotted wings, and it occurs through a wide series of Anophelines, often along with considerable ornamentation of the second type of spotting. As it is seen chiefly (if not entirely) in what I shall show reason to believe are certain primitive groups, and as it is quite distinct from the spotting due to pale and dark scales, and as something very like it occurs in other groups than the Culicidae, I have called this *primitive spotting*. All species of *Myzorhynchus* which I have examined show it, also *Cycloleppteron grabhamii*, Theo. It is very plainly to be seen in *Pt. lindesayi*, Giles, and is prominent in *L. asiatica*, Leicester.

The species *P. atratipes*, Skuse, has an unspotted costa, its palps are unbanded, and it shows very marked and prominent primitive spotting. It clearly cannot be a *Pyretophorus*, and the certainty with which one can speak is a good example of the value of colour markings in systematic work upon the Anophelinae.

Scale clusters. In *Myzorhynchus* and *Cycloleppteron* there is a condition allied to this primitive spotting, in which dark spots on the veins are formed by scales larger than those on the light

areas. Such areas of large scales are seen, especially on the sixth vein, forming the two dark spots characteristic of these genera.

Representation of spots on the lower surface of the wing. The veins of Anophelines carry scales on both surfaces. The character of the scales carried on each depends, however, on the particular vein concerned. In all Anophelines that I have examined, the upper surface of the first, third, fifth and sixth longitudinal veins carries blunt appressed, and what may be described as *flat scales*. On the under surface, all these veins (where they have scales at all) carry quite a different kind of scale, which in most Anophelines is pointed, and projects freely from the vein. The arrangement of scales carried by the second and fourth vein is reversed, flat scales being present on the under and projecting ones on the upper surface. What have been called lateral wing scales are, for the most part, simply the projecting scales on one or other aspect of the vein, as the case may be. As the end of the wing is approached, i.e., about the level of the forks of the second and fourth, the flat scales become increasingly long and projecting, so that in this position both surfaces of the wing carry projecting scales. This accounts for the appearance of the spots in this region which have a blurred look.

Except for a patch of scales towards its free end, or at most, over half its length, the sixth vein is devoid of scales on the lower surface of the wing. The main fifth and the inner part of the branch are similarly bare underneath. The result is that spots on these veins are very distinct and, under an objective, appear to be formed of special small flattened scales. The remaining veins carry scales on both surfaces.

On the whole, the upper surface of the wing is the more heavily scaled, and partly on this account, and partly owing to the preponderance of flat scales, the colour markings are, as a rule, more intensely represented on the upper than on the under surface.

In most Anophelines, allowing for the somewhat more intense coloration of the upper surface and the scale characters noted, the spots on the upper and under surface correspond. But in certain groups there is a marked lack of pale areas on the under surface corresponding with those on the upper, which may reach such an extent that the *under* surface of the wing is practically dark scaled

throughout. A good test of this condition is the third longitudinal, which is most frequently a pale vein. In species where want of correspondence of coloration is marked, the pale, flat scales on the upper surface of this vein will be seen to be flanked by dark lateral scales (i.e., ventral scales), seen through the membrane.

This appearance, called admixture of dark and light scales, has often been referred to, but, so far as I know, no one has previously realised its nature or significance. The group in which dark scaling of the under surface of the wing reaches its most striking development is the *Myzorhynchus* group, including *Cycloleppteron* and *Arribalzagia*.

A rather interesting condition was noted in the halteres of a specimen of *Cycloleppteron mediopunctatus*, which showed, distinctly, the appearance of an unexpanded wing. These structures were covered with snowy white scales on the upper surface, with a central dark spot, but on the under surface were clothed with deep black scales.

The wing of *Myzorhynchella nigra*, Theo., is almost entirely dark scaled beneath, though it has many pale spots on the upper surface; also many pale spots present on the upper surface are lacking on the lower in *P. watsonii*, Leicester (*Myzorhynchus*?) a species allied to *My.* (?) *natalensis*, Hill and Haydon, as well as in *Nm. elegans*, James. *Cellia squamosa*, Theo., and *M. lutzi*, Theo., show a third longitudinal, light above and dark below. *P. atratipes*, Skuse, is another species in which the wing is almost entirely dark scaled beneath, though it carries quite conspicuous pale areas on the second, fourth, fifth and sixth veins above.

In *Pt. gigas* and *Pt. simlensis* the markings are, for the most part, reproduced on the lower surface of the wing.

In *Myzomyia*, *Pyretophorus*, *Nyssorhynchus* and in *Cellia pulcherrima*, Theo, the wing spotting is equally represented on both surfaces, though in some 'dark' *Nyssorhynchus* dusky scales, not definitely dark or light, may be present beneath the third vein.

Admixture of dark and light scales. Apart from the effect of admixture, due to the showing through of dark scales beneath the wing membrane, there is in *Myzorhynchus*, *Cycloleppteron* and *Arribalzagia* a true occurrence of mixed black and pale scales on many of the veins. Such admixture of scales is not seen in the

majority of Anophelines, and does not occur, so far as I am aware, in *Myzomyia*, *Pyretophorus*, *Nyssorhynchus* or *Cellia*.

All the characters so far mentioned must be looked upon as special to certain groups of Anophelines. What one may call the more ordinary Anophelines show neither primitive spotting, nor any degree of unequal ornamentation of the wing surfaces, nor admixture of light and dark scales on the veins, and in them spotting depends entirely upon the existence of definite areas of pale and dark scales on the veins.

Another condition of possible importance, but concerning which my observations are too imperfect to enable me to deal, is staining of the wing membrane in the area of dark spots. This is greatly exaggerated in *Cycloleppteron mediopunctatus*, but is seen in many species.

Nodal Leucogenetic Centres of the Wing

The wings exhibit very clearly a system of leucogenetic centres and residual pigment areas. The leucogenetic centres are of two kinds, those associated with what may be called '*nodal points*' of the wing, and those which I shall call '*internodal*.'

The nodal points in the wing of an Anopheline which have relation to spotting are shown by the small circles in Plate VI, fig. 1. They may be enumerated as follows:—

(1) The apical nodal point at the junction of the first longitudinal vein with the costa (*Ap.*).

(2) The subcostal nodal point at the junction of the subcostal vein with the costa (*S.c.*).

(3) The humeral nodal point where the humeral cross vein enters the costa near its base (*h.*).

(4) Nodal points in connection with the cross veins (C_2-C_5).

(5) Nodal points at the bases of the forked cells (bifurcation of veins two and four).

(6) Nodal points at the junction of all veins and their branches with the wing margin.

There are other points whose nodal origin is possible or probable. These are:—

(7) A point on the first longitudinal vein slightly internal to

the termination of the second longitudinal. If, instead of being joined to the first longitudinal by a cross vein (cross vein 2), the second longitudinal itself formed a direct junction with this vein (*Vide* remarks on wing venation in Section III), it would presumably enter at the nodal point in question. Some authors figure the vein as acting in this way; but I have not found any example of an Anopheline wing showing this arrangement. On the other hand, it appears to occur in some other Culicidae (occasionally in the case of *Theobaldia annulata*). The nodal point in question is an important one, and, as it occurs at the origin of the Sector radius vein, I have called it the *Sector nodal point* (*S*). The nodal point at the entrance of the cross vein actually linking on the Radio-Sector, which is also a very important one in relation to wing markings, may be called the *Accessory Sector* (*S'*).

(8) It is possible that a nodal point occurs on the first longitudinal at *C*.1.'' in the diagram. In Anophelines, no sign of any cross vein exists here, but such a cross vein is commonly seen in Tipulids (*Vide* Plate VI, fig. 2). Another possible nodal point is at *C*.1'. Breaking of the subapical costal spot in *P. costalis*, and some other species, also suggests a point of a nodal character, marked with a (?) in the diagram.

These points behave as nodal points, and it is possible that a study of the developing wing in the nymph may throw light on their origin. But at present their structural origin can only be suspected.

It is noticeable in regard to white areas formed at the nodal points, that they do not show so active a tendency to spread as do those of an internodal character.

Species showing 'dark' or 'light' areas at the cross veins and bifurcations. Certain nodal points may be represented by a leucogenetic centre or by a pigment centre. The nodal points on the costa rarely exhibit the latter condition, but the humeral junction is not infrequently the site of a pigment spot (instead of a pale spot), as in *Cycloleppteron grabhamii*. Still more rarely, the junction of the subcostal vein may be the site of a dark spot, *Cy. mediopunctatus* and *Ar. maculipes*, whilst a dark spot in close association with this junction is seen in *Nm. elegans*, *M. punctulata* and *N. annulipes*, Walker. Such spots form small accessory spots

on the costa (apart from the basal accessory spots to be described later), and are possibly of much greater significance than their mere size might suggest.

The nodal points of greatest importance in connection with their power to be either ' dark ' or ' light ' are those at the cross veins and bases of the cells. In the genera *Myzomyia*, *Pyretophorus*, *Nyssorhynchus*, *Cellia*, and *Neomyzomyia*, the cross veins are always the site of light interruptions. In *Myzorhynchus*, *Patagiamyia*, *Cycloleppteron*, *Arribalzagia*, and *Myzorhnychella* these areas not only are dark, but, when given the chance, exhibit themselves as residual pigment centres. In a great many cases, in addition, they are the site of scale aggregations, giving rise to primitive spots (*Vide* Plate VIII, figs. 1-13).

Nodal points on the costa and first longitudinal. In those species with unspotted costa, we may consider that none of the nodal leucogenetic centres have displayed themselves. This is the typical condition in *Anopheles*, restricting this term to the genus as defined by James (i.e., without a prothoracic tuft). It is also the condition in *Stethomyia*.

The first nodal leucogenetic centre to become evident in all other species, without exception, is the *apical*, giving rise to the condition seen in *Pt. lindesayi* and *P. atratipes*.

The next nodal centre to appear is invariably the *subcostal*. This is the typical condition in *Myzorhynchus*, though in some species (*My. sinensis*, *My. pseudopictus*) the sector spot has also made its appearance on the first longitudinal, but has not spread on to the costa. The same condition is seen in *L. asiatica* and in the related form *My. wellingtonianus*, Alcock.

The third nodal centre to appear as a pale spot is either the *sector spot* or the *humeral spot*, usually together with a spot at the extreme base of the wing and at the (? nodal) point *C*.I.'

If the sector spot becomes prominent without the others there results the *sinensis* type of wing. If the sector spot remains in abeyance and others develop, the *gigas* and *punctipennis* type of wing is produced (Plate VIII, figs. 9 and 4).

So far, all species showing no further elaboration of the costal spotting than that described, have dark cross vein areas, frequently exhibit primitive spotting and a scheme of wing ornamentation in

regard to internodal spots, which is distinct from that seen in *Myzomyia*, *Pyretophorus*, etc.

The sector spot and the (?) nodal spot at *C*.1.″ appear in most cases simultaneously, giving rise to a four spotted costa. The condition in *Cycloleppteron grabhamii* is that shown in *Myzorhynchus*. In the case of *C. mediopunctatus*, the sector spot has included the costa, pale areas have appeared at the base, and the extent of the dark scaled portion is less. In *Arribalzagia maculipes* the dark costal spots are still more sharply differentiated and restricted. Both these greatly modified wings appear to be distinctly a development of the *Myzorhynchus* pattern. In *Myzomyia*, *Pyretophorus*, *Nyssorhynchus*, *Neocellia*, and *Cellia*, the costal pattern, except as the result of occasional individual or specific (?) variation, shows four main spots due to the formation of pale centres at the apical, subapical, subcostal, and sector nodal points, respectively. In the great majority there is also a pale area at the humeral, at the base of the wing, and at *C*.1.′. The accessory sector spot may be blended with the sector spot, merely shortening a little the middle spot as represented on the first longitudinal vein (e.g., *M. culifacies*). It may be lost in the general extension of the sector spot (e.g., certain *Pyretophorus*), or it may form a distinct interruption towards the inner side of the middle dark costal spot, as is the case throughout *Nyssorhynchus*. In most *Nyssorhynchus* a very similar interruption is also seen towards the outer end of the spot. The exact origin of this second interruption is uncertain, but it lies opposite the junction of the third cross vein with the stem of the second longitudinal. Where the inner interruption only is present, I shall speak of the middle costal spot as being 'incompletely broken,' where both interruptions are seen, I shall refer to this spot as 'completely broken' (Plate VIII, figs. 34, 37).

In certain species one or both of the small dark scaled portions on the first longitudinal, separated off by the pale interruptions, may have disappeared. The disappearance of both small spots gives what is frequently spoken of as the 'T' shaped spot of *Pm. rossi*.

The same kind of interruption may occur, but not commonly, on the first longitudinal, under the subapical dark costal spot.

This is very characteristic of *P. costalis*, and is seen particularly in the many-spotted winged species. It can be referred to as a broken subapical spot. (Plate VIII, fig. 25.)

Accessory costal spots. On the costa at the base of the wing are very commonly seen from one to three small dark spots which as a rule are unrepresented on the first longitudinal or even on the subcosta. These may conveniently be termed *basal accessory spots.* In *Myzomyia*, *Pyretophorus*, *Nyssorhynchus*, etc., two such spots are most commonly seen lying on either side of the junction of the humeral cross vein; but three are sometimes present, and not infrequently they may join together among themselves, or, either in part or wholly, be linked on to the inner main costal spot, giving the appearance commonly seen in *M. listoni.* (Plate VIII, fig. 28.)

Another type of basal accessory spot is sometimes seen where the dark area occurs at the junction of the humeral cross vein, e.g., *Cycloleppteron mediopunctatus.*

The number and arrangement of accessory basal spots, or whether any light areas exist at all on this part of the costa, does not seem to be very important.

The selection of the site of junction of the humeral vein for a dark spot is, however, very significant.

Accessory costal spots other than the basal accessory spots are found in certain cases. These appear to be of considerable significance, and their exact site is very important. The mode of formation and homology of such spots in different species can be traced. In *Ch. kochi*, Donitz., a prominent accessory spot is seen on the costa between the inner and middle dark costal spot. The nature of this spot is clearly shown by a study of the wings of *P. watsonii*, Leicester, and of *My. natalensis*, Hill and Haydon. The wing of *My* (?) *natalensis*, as shown in the photograph given by its describers, is a beautiful demonstration of the typical subdivision of the costa into dark spots by the appearance of the apical, subcostal, sector, accessory sector and *C.*I." nodal leucogenetic centres. In the species *P. watsonii*, Leicester, the type of which is in the British Museum, a very similar arrangement of spotting is shown, but the accessory sector spot has developed to such an extent as to reach the costa, forming a small detached dark spot clearly identical with that seen in *Ch. kochi.* This fact is the

more interesting in that such a spot is not common in Anophelinae, and that it is for the most part seen in the many-spotted-winged species, e.g., *Nm. leucosphyrus*, Donitz. *P. watsonii* and *My. natalensis* show therefore very curious affinities; showing an approach to *Myzorhynchus* they so far differ as to have been excluded, or to have been regarded as doubtfully belonging to this genus, by their describers; they distinctly recall some *Patagiamyia* characters and as pointed out they have some unusual points in common with the many-spotted-winged group and with *Ch. kochi*, Donitz, which species it should be remembered has *Myzorhynchus*-like wing scales.

Bridging of costal spots on the first longitudinal. Certain species, including *C. squamosa*, Theo., *C. albimana*, Wied., and *N. fuliginosus*, Giles, show a bridging of certain of the pale costal areas by dark areas on the first longitudinal. The same feature is displayed in *Myzorhynchella nigra*, Theo. The species *Myzorhynchella nigra* shows a four-spotted costa; it has a wing with a fair number of markings above, but almost entirely dark-scaled below; the cross vein nodal points are dark; it has prothoracic tufts; there can be little doubt but that it is related to *Myzorhynchus*. If, as seems possible, *C. squamosa*, which exhibits distinct *Myzorhynchus*-like affinities, really descended from a primitive *Myzorhynchus*-like ancestor, one would expect this form to be something like *Myzorhynchella*, though the evidence for such a descent, and for that tentatively suggested for the *elegans* group, is very slender.

Nodal points on wing margin. When pale spots display themselves in this situation they affect not only the veins but also the wing fringe, forming 'pale fringe spots.' The amount of possible spread of these spots, like most other spots of nodal origin, is small.

Internodal Leucogenetic Centres of the Wing

The internodal spots appear as a rule almost exactly in the centre of long stretches of dark vein lying between nodal points, and the order of appearance of the internodal spots has a distinct relation to the length of the dark area concerned. The internodal points in the scheme of coloration shown by *Myzomyia*, *Pyretophorus*, *Nyssorhynchus*, etc., are:—

(1) One on the upper branch of the second (2^1). Two on the lower branch (2^2 and 2^3). These are usually so placed that they alternate, as shown in the diagram, and the result is that the limbs of this forked cell are *asymetrically* marked.

(2) On the shaft of the third vein. Whether one or two centres are here concerned is not quite certain, but I am inclined to think that there are two which very readily blend (*vide* appearance shown in some species).

(3) One on the upper branch of the fourth vein (4^1). One on the lower branch (4^2). In this case the branches are marked symmetrically, though owing to the slope of the wing edge the actual position of the spots is not quite opposite one another.

(4) One on the upper branch of the fifth, i.e., between the nodes *C*.5. and 5′. Two on the main branch of the fifth, one above and one below the junction of the branch.

(5) Two on the sixth, dividing this vein approximately into three portions.

Not only have these internodal centres a nearly fixed position, but they have an almost fixed order in which they become apparent by the development of pale spots.

The internodal spots, once they appear, tend to extend very rapidly along the veins, causing much more extended pale areas than do the nodal points.

The first spot to appear is 5^1 on the main fifth beyond the fork. In certain species this is the only internodal spot present (e.g., *funesta* var. *umbrosa*). In *M. culicifacies*, *M. nili*, etc., this is also the only conspicuous spot present.

The next spot to appear is generally one of those on the third vein, giving rise to a variety of *M. culicifacies* and *M. funesta* var. *subumbrosa*, with more or less pale third longitudinal. In association with this appears a spot on the upper portion of the fifth (5^2), and a pale area at the base of the fourth longitudinal (4). The wing at this stage of ornamentation is that seen in the lighter-winged *Myzomyias*, i.e., *M. listoni*, *M. funesta*, etc. The only *Myzomyia* (excluding certain forms previously mentioned) which shows any further ornamentation is *M. albirostris*, Theo. (*M. aconita*, Donitz?), a peculiar species in many respects, including the larval characters.

With a further degree of lightening of the wing, additional spots

appear at 5^2 on the branch of the fifth, and on the forked cells, 2^3 being a little later in appearance than the others. Whilst these areas have, as it were, only just appeared, the ones previously noted have spread considerably, so that as a rule the third vein is completely pale, for example, in any species showing a pale spot at 5^2.

The scheme so far developed is that seen in a number of species of *Pyretophorus;* though in other species of this genus an additional spot (6^2) has appeared.

The nodal points on the edge of the wing behave in concert with this progression. In the wings showing only 5^1 the fringe spots are generally deficient (e.g., two in *M. culicifacies*). In association with the appearance of the nodal centres 3 and 5^2, fringe spots are generally found at all veins except the sixth. The nodal point at the termination of this last vein only occurs as a rule after 6^2 has appeared. At about the same time another (?) nodal spot not previously represented forms a pale area on the first longitudinal, opposite cross vein 3. It is this which gives the double break in the middle costal spot so characteristic of *Nyssorhynchus*, etc.

Whilst no further internodal spots appear, the wing still continues to show modifications owing to the encroachment upon, and in some cases obliteration of, residual pigment areas. The more important changes produced by this extension of the internodal centres are (*a*) the blending of 5^1 and 5^2, thus producing the commonest condition of the fifth vein in Anophelines (*vide* Plate VII, fig. 4); (*b*) the obliteration of one or both of the small spots on the first longitudinal under the main spot (points of the 'T' in *rossi*); (*c*) obliteration of one of the dark spots on the sixth vein, either the upper or the middle one as in some species of *Cellia*; (*d*) obliteration of one or more of the small dark spots on the forked cells (usually the first to go is $2''\,b$, the next most usually $2'\,b$ or $4''\,b$); (*e*) the obliteration of the dark spot $4r$.

In addition to mere extent of white involved there is a difference in the intensity of the colour. Roughly speaking, *Myzomyia* and *Pyretophorus* show *pale* but not markedly *white* areas. *Nyssorhynchus* and *Cellia* exhibit a striking dead-white ornamentation.

In *Myzorhynchus*, *Cycloleppteron*, *Patagiamyia*, etc., some of the internodal centres appear to be the same as certain of those

described, but there is no general correspondence in the scheme of coloration. Also in this group there seems to be a much less orderly sequence of such pale areas as do appear. The whole effect of the ornamentation is of course modified by the fact that the cross vein nodal centres are dark.

A group showing light nodal centres, but having a wing ornamentation scheme absolutely without relation to that of *Myzomyia*, etc., is the group Donitz[4] has drawn attention to as markedly peculiar owing to the character of the wing spotting. The most striking character of the wing in this case is the extraordinary multiplication of spots due to alternate short lengths of dark and light scaling on the veins. Practically none of the internodal areas seen so regularly throughout *Myzomyia*, *Pyretophorus*, etc., can here be made out. The sixth vein shows from four to six dark areas, the main fifth at least three interruptions on each portion of the stem and on the branch respectively. The branches of the second and fourth veins have each a double interruption. The third, instead of being throughout most of its length either dark or pale, is broken up into alternate light and dark areas.

Summarising what has been detailed above, we can say that there are three absolutely distinct colour schemes on which the spotting of wings of Anophelines is modelled. These are:—

(1) Scheme A. The scheme on which the wings of most ordinary Anophelines are spotted, characterised by the very regular appearance in a definite sequence of certain internodal pale spots and by the cross veins and bifurcation nodal points being pale.

(2) Scheme B. A less regular scheme shown by *Myzorhynchus*, *Cycloleppteron*, *Patagiamyia*, etc., in which the nodal points at the cross veins and bifurcations are dark.

(3) Scheme C. A scheme characterised by multiple spotting of the veins and the occurrence of more than three dark spots on the sixth.

These three schemes not only seem of the most fundamental importance, but as displayed in the great majority of species of Anopheles can be distinguished at a glance.

Variation in Colour Marking shown by Individuals of the same Species

In any attempt to utilise colour markings as a basis of classification the question of individual variation must be considered. It may be objected that such variation as is displayed in many species will prevent use being made of colour markings. This I think is not necessarily so, because variations shown by individuals of the same species have only a certain latitude, and with a correct appreciation of what a given variation really amounts to one is able to allow for variation in classification. The appearance of a new centre out of its order, or the change of the markings over several steps of normal colour marking development, would be a much more serious matter than the final extinction of a disappearing pigment area or the appearance of a pale centre already on the point of appearing. In this respect I believe a knowledge of the principles underlying colour marking will give an added importance to observations upon individual variations. Certain stages seem distinctly unstable, others much more stable. The knowledge as to the relative stability of different conditions cannot but be of value in deciding whether certain forms are species or varieties.

III. VARIATION IN STRUCTURE OTHER THAN THAT CONNECTED WITH SCALES

Very few structural variations other than those connected with scales are mentioned in descriptions of species of Anophelinae. This is in itself rather suggestive that such variations as do occur are not of an obtrusive nature. So far as I can ascertain the following are almost the only points in which different species of Anophelines differ from one another in respect to structure.

General shape of body. Donitz[4] has called attention to certain characters of the head and the shape of the thorax in Anophelinae. These require fresh material for proper study, and my observations in this respect do not add anything to what was previously known.

Closely associated with the general shape of the body is the attitude adopted by the species when at rest. The most importance attaches to the possession of a '*culex*'-like attitude. This attitude seems to be a definite character of *Stethomyias*. *M. culicifacies*,

Giles, and *Chagasia fajardoi*, Cruz, are the only species apart from this group which, so far as I know, have been described as adopting this attitude. The '*culex*' attitude in the former is not nearly so striking as in the case of *S. aitkeni*, which superficially almost exactly resembles some long-legged *Aedine* mosquito.

Apart from the assumption or not of the '*culex*' attitude, there are marked differences in the angle made by different species with the surface on which they are at rest. Observations in this respect are too limited to allow any general conclusions being drawn as to any possible significance attitude may possess in respect to generic affinities.

The antennae. In the female antenna the arrangement of the segments in respect to their length is somewhat peculiar. Following upon the large globular basal (second segment according to Nuttall) is a rather long segment. The next segment is in most species not only the shortest in the whole antenna, but is often almost or quite globular. In *My. barbirostris*, *My. umbrosus*, *My. paludis*, *My. sinensis* and *C. grabhamii*, though this segment is still the shortest in the antenna it is not nearly so disproportionately shortened, nor has it the same globular appearance as in most Anophelines. The segment is also rather longer than usual in *P. jeyporensis*, James.

The male antenna possesses a very striking structural peculiarity in the great length of the two terminal segments. But so far as I could see the relative lengths of these structures exhibited very little change in different species, nor do the antennae of different species differ noticeably in other respects.

Female palpi. The number of articulated segments in the female palps in Anophelines is four. There is, however, a vestigial prominence carrying tufts of scales at the extreme base of the palps which probably represent a fifth segment.

Distinct variations occur in the relative length of the different segments. In this respect two types of palps can be distinguished, which I have termed respectively *orthodactylous* and *heterodactylous*.

In the first or *orthodactylous* palps the segments observe a kind of proportionate decrease in size as the apex of the organ is approached. In the very orthodactylous genera, such as *Myzorhynchus*, the terminal segment may be as much as two-thirds the length

of the penultimate. One may define an orthodactylous palp as one in which, whilst the segments one and two keep their proportionate length (they only vary to any extent in *Stethomyia*), the last segment is not less than half the length of the penultimate segment.

In the second or *heterodactylous* type of palp there is a marked want of regularity in the decrease exhibited by the segments. Of heterodactylous palps two distinct types are encountered, due to the disproportionate length of the preapical and second palpal segment respectively.

Great increase in the length of the preapical segment is seen in both *Myzomyia* and *Pyretophorus*, the apical segment being a third or even a quarter of the preapical (Plate V, fig. 10). Such a marked structural variation in a sub-family otherwise showing very few changes in structure must have considerable significance. Species with distinctly orthodactylous palps now included in these genera, e.g., *P. costalis*, Loew., would seem to require separation.

The second type of heterodactylous palps occurs in *Stethomyia*. In this case the second segment is very long and the third disproportionately short (Plate V, fig. 16). This variation is the more remarkable in that amongst other Anophelines any marked change in the relative length of the first and second segments is conspicuously absent. This type of palp cannot be overlooked, if attention is directed to it, and it would seem in itself a sufficient and very good generic character. As previously mentioned, *S. corethroides* shows the linear head scales characteristic of *S. aitkeni*. It also shows the peculiar modification of the palps mentioned above. A similar condition of the palp is figured by Theobald for *S. nimba*.

Expressing the relationship of the terminal to the penultimate segment in a decimal, one can obtain, when dealing with orthodactylous palps or with the first type of heterodactylous palp, a very useful index. What may be called the palpal index for a number of species is given below. This index can be determined very accurately from balsam-mounted specimens by direct measurement with an eye-piece micrometer, but better by making camera lucida drawings, taking care to avoid any possibility of distortion. Those indices given in the table where no other remark is made have been determined in this way and checked by direct measurement.

Species	Palpal index	Source of information
My. barbirostris	0·61	Mounted specimen
My. umbrosus	0·69	,,
My. mauritianus	0·55	,,
Cy. grabhamii	0·70	,,
A. maculipennis	0·57	,,
A. bifurcatus	0·50	,,
Pt. lindesayi	0·71	,,
M. funesta	0·32	,,
P. nursei	0·32	,,
P. cleopatrae	0·35	,,
P. jeyporensis	0·32	,,
Pm. rossi	0·50	,,
P. costalis	0·75	,,
N. fuliginosus	0·60	,,
Ne. fowleri	0·62	,,
N. maculatus	0·50	,,
C. pulcherrima	0·54	,,
C. squamosa	0·68	,,
C. albimana	0·79	Specimen not mounted
M. aconita	0·43	From Donitz's figures
M. bebes	0·33	,,
M. deceptor	0·59	,,
Nm. leucosphyrus	0·68	,,
M. punctulata	0·63	,,
M. tenebrosa	0·73	,,
N. maculatus	0·50	,,
N. leucopus	0·46—0·57	,,
P. cinereus	0·37	Photo by Hill & Haydon
P. ardensis	0·75	,,
My. natalensis	0·76	,,

Given a good photograph, one can usually make a rough determination of the palpal index; very often, however, a glance will tell one whether a particular palp is orthodactylous or distinctly heterodactylous.

In the drawings given in the Plate the palps of different species have been drawn by means of a Zeiss adjustable lens (*a**), so that they are shown of the same size (not scale). This shows very clearly the variations in the proportions of the different segments. The extremely low index of *Myzomyia* and the *nursei* group of *Pyretophorus* is practically diagnostic.

The male palpi. These show a change more or less analogous to that seen in the female, the palp being more abruptly clubbed and the last two segments taken together measuring proportionately less in the heterodactylous species than in others.

The wings. On the whole the structural characters of the wings are more interesting from their fixed character than for the variations they exhibit. Two conditions have, however, been made use of as variables by Theobald and others. These are the positions of the cross veins and the length of the forked cells. On these subjects I have nothing new to add.

Much more important from our present point of view are certain points connected with the homology of the wing venation in Anophelines. According to Comstock and Needham the generalised insect pattern of wing venation shows the following main longitudinal vein systems, the branches of which are represented more or less closely by tracheal branches which precede them in development:—

(1) The costa.
(2) The subcosta.
(3) The radial system.
(4) The medial system.
(5) The cubital system.
(6) Three anal veins.

The subcosta is normally a bifurcate vein, the branches of which enter the costa at two points. In many forms the lower branch becomes fused with the radius for a portion of its length, so that there appears to be (1) a branch joining the terminal portion of the

subcosta with the radius, and (2) a branch going from the radius to the costa. The auxiliary or subcostal vein in Anophelines is an unbranched vein entering the costa a little beyond its middle. It seems quite clear that it is homologous with the subcosta of the generalised pattern, and it shows the *humeral cross vein* joining it and the costa near its base, which is a feature of the generalised wing.

The radius in its typical development consists of an unbroken vein R_1, passing from the base of the wing to the neighbourhood of the apex (Plate VI, fig. 2). Posteriorly to this there arises a branch (the *radio-sector*) which sub-divides into two branches R_{2+3} and R_{3+4}, each of which again sub-divides, forming four terminal branches R_2, R_3, R_4 and R_5. Between the lower branches of the radio-sector (R_{4+5}), and the medial system is the second of Comstock and Needham's cross veins, the *radio-medial*. Nuttall and Shipley consider the first longitudinal vein to be R_1, the branches of the second longitudinal to be R_2 and R_3 respectively, whilst the third longitudinal is the combined R_{4+5}. Following this homology the radio-sector will be that portion of the second longitudinal between its origin and the origin of the third vein, i.e., where the supernumerary cross vein enters it.

Some figures show the second longitudinal as a branch of the first. In reality, though it is joined near its origin to the first longitudinal by a cross vein, the vein itself continues past this a short distance to end on the wing membrane, as noted by Nuttall and Shipley. Presuming the second longitudinal to be homologous with the radio-sector, its true line of departure would be a little internal to the cross vein, a point we have referred to when discussing the formation of wing spots.

The division of the radio-sector into R_{2+3} and R_{4+5} would, following Nuttall and Shipley's homology, occur at the so-called supernumerary cross vein. Some authors figure the third vein as arising directly from the second, but this is not actually the case, and the junction is brought about by means of a cross vein, the vein itself continuing slightly beyond this to lose itself on the wing membrane.

The cross vein joining the third with the stem of the fourth vein would appear to be one of Comstock and Needham's original cross

veins (radio-medial). The fourth longitudinal would appear to represent the median, the fifth longitudinal the cubitus, and the sixth the first anal.

The only cross veins in Anophelines actually represented as cross veins in Comstock and Needham's generalised nomenclature are (1) the humeral, (2) the medio-radial, (3) the radio-cubital, these being respectively the humeral, the mid and posterior cross veins of Theobald. But there are, as we have seen, additional cross veins which are quite indistinguishable structurally from the first-mentioned, and one of these (that linking the third with the second vein) is called the supernumerary by Theobald. The cross vein at the origin of the second vein, though noted by Nuttall and Shipley, has not received a name. Since it links up the radio-sector to R_1 it might appropriately be called the radio-sector cross vein. I would suggest, however, the use of the terms first, second, third, fourth and fifth as applied to the cross veins which makes no attempt, as is very imperfectly done at present, to denote their homology. It seems absurd to term the longitudinals by numbers and the much less important cross veins by a hybrid nomenclature only partially descriptive. The omission of the radio-sector cross vein, which is every bit as important as the others, is also absurd. In the following pages I shall refer to the cross veins according to the following plan, which has the merit that each vein has the same number as the longitudinal vein immediately behind it.

Homologous nomenclature	Present nomenclature	Suggested name	Position
Humeral	Humeral	Humeral or first	Joins costa to subcosta at base
(Radio-sector)	——	Second	Links on second vein
(Lower radial)	Supernumerary	Third	Links on third vein
Radio-medial	Mid-	Fourth	Joins fourth vein to third
Medio-cubital	Posterior	Fifth	Joins fifth to fourth

On the system suggested the cross veins entering any given vein can be at once named, or the veins that any cross vein affects be at once called to mind without effort.

IV. STRUCTURAL VARIATION IN THE IMMATURE STAGES

A considerable number of larval forms have now been described, and it seems possible to make some tentative use of the facts gathered in attempting to ascertain the affinities of species. In particular the variations in the antenna, i.e., whether it carries a branched hair or not, and the occurrence in certain species of the lanceolate type of palmate leaflet would seem to be significant. Variations in the frontal hairs and other features have been described for a number of species, but the application of such variations to classification is less obvious.

Species with a branched antennal hair. So far as I have been able to ascertain, the following species possess a well marked or vestigial branched antennal hair.

A. maculipennis, Meig. Noted by Hill and Haydon.

A. bifurcatus, Linn. *Vide* Plate.

Pt. lindesayi, Giles. Stephens and Christophers.

A. crucians, Wied. Smith.

Pt. punctipennis, Say. Smith.

Pt. simlensis, James. James.

My. sinensis, Wied. Stephens and Christophers.

My. barbirostris, Van der Wulp. Stephens and Christophers.

My. paludis, Theo. Hill and Haydon.

Chagasia fajardoi, Lutz. Shown in drawing of larva by Silva.

The larval antenna of *Cy. grabhamii*, Theo., is figured by Theobald without a branched hair. This structure can, however, be very readily overlooked if attention is not especially directed to it, and the observation requires confirmation. It is interesting to note that no branched hair is present in *My. natalensis*, Hill and Haydon.

Species with lanceolate type of palmate hair leaflet. The type of leaflet seen in *Myzorhynchus* is not uncommon. The following species have been described as showing lanceolate and serrated palmate leaflets.

A. maculipennis, Meig. Hill and Haydon.

A. bifurcatus, Linn. *Vide* Plate.

My. barbirostris, Van der Wulp. Stephens and Christophers.

My. sinensis, Weid. Stephens and Christophers.

My. paludis, Theo. Hill and Haydon.

Cy. grabhamii, Theo. Theobald.

C. albimana, Wied. Graham.

C. squamosa var. *arnoldi*, Steph. and Christop. Newstead and Carter.

C. albipes, Theo. Low.

Forms showing an approach to this type of leaflet, but in which the leaflet is drawn without serrations and as of a simple fusiform shape.

C. squamosa, Theo. By Hill and Haydon.

Myzorhynchella nigra, Theo. Silva.

Myzorhynchella parva, Chagas. Silva.

Stethomyia culiciformis, James. James.

Forms showing an approach to this type of leaflet in the serrations at the shoulder of the leaflet are more drawn out than in ordinary Anophelines has been noted in:—

My. (?) *natalensis*, Hill and Haydon. By these authors.

Pt. lindesayi, Giles. Stephens and Christophers.

S. aitkeni, James. By this author.

Nm. elegans, James. By this author.

P. ardensis, Theo. Hill and Haydon.

Hill and Haydon figure *N. pretoriensis* with leaflets of this character, but in the photograph accompanying their paper the condition is not so clearly shown.

The extreme degree of branching of the external frontal hairs characteristic of *My. sinensis* is also shown by *My. paludis* (Hill and Haydon). This character may therefore be reasonably taken as one of the features of *Myzorhynchus*. Its absence in any given species would draw attention to doubtful generic position. Marked feather-like branching resembling that in *N. fuliginosus*, Giles, is also seen in *C. squamosa* and in *C. pulcherrima*. The following tabular statement gives, as far as the information available allows, the chief larval characters of a number of species *arranged in the order of their apparent phylogenetic significance.*

Variations in the nymphal structure of Anophelines. Very little variation can be detected in the nymph of different species. There is, however, a slight but appreciable difference between the nymphal trumpets of *My. sinensis*, *My. barbirostris*, *Pt. simlensis*, *A. maculipennis* and *A. bifurcatus* and those of Anophelines

TABLE showing larval characters in order of apparent phylogenetic significance

	Branched hair on antenna + = present, O = absent.	Palmate leaflets lanceolate + + , showing tendency as in lindesayi +	Palmate leaflets fusiform.	Palmate leaflets with ordinary longish filament.	Palmate leaflets with stumpy filament.	External frontal hair branched as in *Sinensis* B, as in *fuliginosus* bb, otherwise distinctly branched b, simple or with fine lateral branches S.
Stethomyia aitkeni	O	+				S
,, *culiciformis*	O		+			S
Anopheles maculipennis ...	+	+ +				B
,, *bifurcatus*	+	+ +				S
,, *eiseni*	+	?				S
,, *crucians*	+	?				S
Patagiamyia lindesayi	+	+				S
,, *simlensis*	+	?				S
,, *punctipennis* ...	+	?				S
Myzorhynchus sinensis	+	+ +				B
,, *barbirostris* ...	+	+ +				B
,, *paludis*	+	+ +				B
Cycloleppteron grabhamii ...	?	+ +				S
Chagasia fajardoi	+			+		
Myzorhynchus (?) *natalensis* ...	O	+				b
Neomyzomyia elegans	O	+				S
Cellia squamosa var. arnoldi ...	O	+ +				bb
,, *squamosa*	O		+			bb
,, *albimana*	O	+ +				
,, *albipes*	O	+ +				b
,, *pulcherrima*	O			+		bb
Pyretophorus ardensis	O	+				
Myzorhynchella nigra			+			
,, *parva*			+			
Nyssorhynchus fuliginosus ...	O			+		bb
,, *nivipes*	O			+		bb
,, *jamesi*	O			+		b
,, *maculipalpis* ...	O			+		b
,, *pretoriensis* ...	O					S
,, *maculatus* ...	O				+	S
,, *theobaldi* ...	O				+	S
,, *karwari* ...	O					
,, *willmori* ...	O				+	S
Christophersia kochi	O				+	S
Myzomyia culicifacies	O			+		S
,, *listoni*	O			+		S
,, *funesta*	O			+		S
,, *turkhudi*	O				+	S
,, *albirostris*	O					b
Pyretophorus cinereus	O			+		S
,, *nursei*	O			+		S
Pseudomyzomyia rossi	O			+		S
,, *ludlowi* ...	O			+		S
Pyretophorus jeyporensis ...	O			+		b
,, *costalis*	O			+		S
Cellia jacobi	O			+		S

belonging to the genera *Myzomyia*, *Nyssorhynchus*, etc., those of the former being more triangular in shape and less scoup-like than those of the latter. (*Vide* Plate V, figs. 1 and 2.)

V. VARIATION AS DISPLAYED BY SCALES

A detailed account of scale structure would be foreign to the purpose of this paper. But it is necessary in connection with other variable features to summarise briefly the main facts regarding scale variations in Anophelines.

This is the more necessary in that it seems to be taken for granted that the only possible classification of the Anophelinae based on scales must be that actually employed by Theobald. That this is a very limited view to take must be granted by all who have paid close attention to scale characters. It may be found that other scale characters not perhaps so conspicuous as those so far employed may be of much greater use than has been suspected. It will be useful therefore, even if the subject is only rapidly passed in review, to indicate the broader principles of scale structure variation. In doing so I shall take the opportunity of making certain revisions in regard to the position certain species now occupy, positions which it would be unfair later on to consider as showing a discrepancy between the results of scale character and colour marking classifications.

Head Scales. Marked variations in the head scaling are restricted to a very few groups. The occurrence of linear head scales has been used by James[1] to differentiate the genus *Neostethopheles* (type *N. aitkeni*, James). This genus is clearly the same as Theobald's *Stethomyia* (type *S. nimba*, Theo.), in which the head scales are also linear. An examination of specimens of *A. corethroides*, Theo., in the British Museum shows that this unspotted winged species from Australia has linear head scales, and should come in the genus *Stethomyia* and not in *Anopheles* (vide also palpal characters). *A. immaculatus*, Theo., and *A. smithii*, Theo., have head scales of the ordinary expanding type.

Scaling of the palps. In some species the scales over most of the palps are of small size and relatively appressed. In such

species the palp has a smooth thin appearance. In others the scales are long and outstanding, giving to the palp a more or less shaggy look. Difference in this respect, though not used in the definition of generic groups, is by no means unimportant. One would view with suspicion any supposed *Cellia* having smooth thin palps, or a supposed *Myzomyia* with shaggy palps. *Pm. rossi*, Giles, a species with shaggy palps until recently termed a *Myzomyia*, is now known not to conform in scale characters with the definition of this genus; presumably the related species *M. indefinata*, Ludlow, *M. ludlowi*, Theo., *M. mangyana*, Banks, are also rightly excluded. The species *M. lutzi*, Theo., at present the only South American representative of *Myzomyia*, has shaggy palps with a quite different type of ornamentation to that seen in any other *Myzomyia*, and the correctness of the present position of this species must be considered as problematical.

Between what may be called the *superpictus* group of *Pyretophorus*, which has very long thin palps, and *P. costalis*, Loew., there is also a very marked difference in this respect, which is accompanied by marked structural as well as important scale distinctions.

Antennal Scales. One of the characters of the genus *Calvertina* (*C. lineata*, Ludlow) is the presence of outstanding scales on the second segment of the antenna. I have not seen a specimen, but otherwise the scale characters from the description seem very like those of *Nyssorhynchus*. The markings also read very like those of *N. fuliginosus*, James and Liston.

Prothoracic Lobes. James (1) has called attention to the importance of the *patagia* or prothoracic lobes from the point of view of classification, and his separation of *Patagiamyia* (type *Pt. gigas*, Giles) from *Anopheles* is based on the presence in the former of a well-marked tuft of outstanding scales on these organs. Using this feature one can distinguish as *Patagiamyias*, in addition to those noted as belonging to the genus by James, *A. punctipennis*, Say., and *A. smithii*, Theo., both of which have prothoracic tufts.

Judging from the light thrown by colour markings, a tuft of scales on the prothorax is an extremely important character. Unfortunately it is only in a proportion of species that the

description given notes the presence or absence of this character. A prothoracic tuft appears to be of general occurrence in *Myzorhynchus* (*barbirostris*, *umbrosus*, *sinensis*, *mauritianus*), there is a tuft in *Cycloleppteron grabhamii*, in *Arribalzagia maculipes* and in *Myzorhynchella nigra*. The tuft characteristic of *Patagiamyia* is present also in *Lophoschelomyia asiatica*, Leicester. *Myzorhynchus wellingtonianus*, Alcock, and *Feltinella pallidopalpi*, Theo., all of which species would appear from their colour markings to be related to *Pt. lindesayi*, Giles. A tuft is present in *Christophersia kochi* = (*Cellia kochi*, Donitz.), *Neomyzomyia elegans*, James, *Cellia squamosa*, Theo., and curiously enough in *P. costalis*, Loew. On the other hand, a large series of species do not show this structure.

At present, information is too scanty to enable any hard and fast use to be made of the prothoracic scaling in classification, but the table accompanying this section indicates approximately and very tentatively the possible significance of the occurrence of a tuft in this situation.

Mesothoracic Scaling. The presence or absence of broad scales on the thorax has been used along with other characters to differentiate a number of genera. Restricting observations to certain groups, the presence or absence of broad mesothoracic scales seems to serve the purpose of defining genera fairly well. By its aid *Myzomyia* is fairly clearly marked off from *Pyretophorus*, etc., and if the shape and character of the scales is also taken into account, this latter genus can be quite well differentiated from *Nyssorhynchus*.

Extending our observations, a good many reasons for restricting the significance of thoracic scaling become apparent, and any groups in which primary divisions were formed on this character would be very heterogeneous.

The great difficulty in scale structure is to know what significance can be attached to particular variations. Thus in comparing *Pm. ludlowi*, Theo., and *P. costalis*, Loew., which differ very slightly in markings, character of the palps, etc., one does not know whether the presence in the latter of mesothoracic scaling is to be taken as sufficient proof that they are unrelated or not. The same difficulty occurs in the case of abdominal scaling

TABLE shewing tentative grouping on general scale characters.

		Tuft on prothorax		No tuft on prothorax		
		Broad scales on mesothorax	No broad scales on mesothorax	No broad scales on mesothorax	Broad scales on mesothorax	
Head scales linear	Abdomen devoid of scales			Stethomyia (palps thin)		Wing scales moderately broad or narrow. Palps may be thin
Head scaling of ordinary type	Abdomen devoid of scales		Patagiamyia (Palps thin) Feltinella *My. natalensis*	Myzomyia Anopheles (Palps thin)	Pyretophorus (*nursei* group) (Palps thin)	
	Abdomen with a few scales on last segment, no tufts	*P. costalis* (wing scales not very broad)	Neomyzomyia	Pseudo-myzomyia		
	Scales on several segments, no tufts		Lophoschelomyia (Manguinosia) (?) (Kerteszia) (?)		Nyssorhynchus Neocellia	
	A ventral tuft often present with or without other scales		Myzorhynchus Cycloleppteron			Wing scales very broad. Palps shaggy
	Many ventral tufts	Christophersia				
	Lateral tufts	Arribalzagia (*C. mediopunctatus*) *C. squamosa*			Cellia (some species)	
Head scales appressed	Abdomen without scales	Myzorhynchella				
Head scales narrow curved	Abdomen without scales	Chagasia				
Arrangement of scales peculiar	Abdomen with long lateral tufts		Christya (Peculiar genus narrow curved scales on prothorax)			

For most part wing scales very broad (Myzorhynchus-like)

in the species *Ne. willmori*, James, and *N. maculatus*, Theo., which it is practically impossible or very difficult indeed to distinguish by any other characters than that one has a very much more scaly abdomen than the other.

Metathoracic Scaling. Limited observations only, which have not shown much promise, have been made in this respect. It was thought that since the metathorax represented a primary division of the thorax such scaling as was present might have more significance than its mere extent or conspicuousness might at first suggest.

The *halteres* which spring from the metathorax, as one might expect from their representing an undeveloped pair of wings, carry scales. Usually the halteres are quite ball like, but in certain species (e.g., *Arribalzagia maculipes*) the wing-like character is much more apparent (Plate VIII, fig. 39).

Wing Scales. Considerable differences occur in the shape of the wing scales. The most noticeable variation is in the breadth of the scales. There is also a difference pointed out by James, in that whilst in some species the scales are broadest in the middle (elliptical), in others they are broadest towards the free end (oblanceolate). This character serves, according to James, to distinguish between the genera *Anopheles* and *Myzomyia*.

Leg Scales. The genus *Lophoschelomyia* has been formed for *L. asiatica*, Leicester, which has outstanding scales on the femur. Recently another species showing the same appearance, *My. wellingtonianus*, Alcock, has been described. Examination of specimens in the British Museum shows that both these species are not only probably related to one another, but have affinities with *Pt. lindesayi*, Giles. *Pt. lindesayi* is at once differentiated by possessing a very striking white band about the middle of the femur. So far as one could see, this colour peculiarity was a pure idiosyncrasy of the species. But the presence of a white band in connection with the structural peculiarity of a prominent scale tuft on the femur in *L. asiatica* and *My. wellingtonianus* suggests that the band in *lindesayi* was once also accompanied by scale tufts. The band is not quite in the same position in *L. asiatica* as in *My. wellingtonianus* but its occurrence along with a scale tuft is unlikely to be a mere coincidence. The three species in question in any case appear to be closely related, and if the genus *Lopho-*

schelomyia is retained, it should probably include *Pt. lindesayi* (vide diagrams of wing markings, Pl. VIII, figs. 1, 3).

Abdominal Scales. Abdominal scaling, as pointed out by James, shows two distinct characters depending upon whether the scales project to form tufts or not.

Tufts are usually, but not always, associated with ordinary scaling. They may be lateral or ventral. The genus *Cellia*, as is well known, shows a marked development of *lateral tufts;* the same condition is shown in *Arribalzagia maculipes*, Theo. The peculiar species *Ch. kochi*, Donitz., shows a marked development of *ventral* tufts, whilst the (apparently) totally unrelated *Myzorhynchus* group has one such tuft (not, however, present in all species).

Pyretophorus is defined as having no scales on the abdomen. On close inspection I find that there are a few scales in *P. costalis*, Loew., especially in the male, the appearances being much like those seen in *Pm. rossi*, Giles. *P. costalis*, Loew., is the type of the genus, so that the definition of *Pyretophorus* (abdomen without scales) now held will not serve. The group of mosquitos like *P. nursei* seem to have a quite scaleless abdomen.

A common condition is that in which the last two or three segments only carry scales. This is best known in *Nyssorhynchus*, but it also occurs in *Lophoschelomyia*, *Manguinosia*, and *Kerteszia*.

In *Cellia*, *Neocellia*, *Christophersia*, and *Arribalzagia* the abdomen carries large numbers of scales.

In the table I give a quite tentative and provisional grouping based on scale structure, adopting as broad an outlook as possible. It will be noticed that I have given great prominence to the condition of the prothoracic lobes. It is possible that this may require modification, but at present all my observations have gone to show the extreme significance of the presence or absence of a tuft of scales on the prothorax.

VI. CLASSIFICATION, PHYLOGENY AND GEOGRAPHICAL DISTRIBUTION OF THE *ANOPHELINAE*

A tabular statement of groups as characterised, and to some extent defined, by structural characters and colour markings accompanies this paper. Though scale characters are given in this, they only enter very occasionally into the formation of the groups, and are purposely placed in parenthesis in order that their close agreement with the general scheme of classification given may be apparent. It will be seen that in the main Theobald's genera stand out clearly. Consideration of other characters, however, modifies Theobald's divisions as follows:—

(1) It shows certain genera to be composite.

(2) It groups the genera together so that one has some realisation of their zoological value.

(3) It shows that it is not impossible that a system of quite good genera, more or less on Theobald's line, could be with advantage retained, and that it is premature to resort as yet to the abolition of this grouping.

I do not propose, in the present paper at least, to offer any new system of nomenclature, which would require, in order not to add to to the confusion already too apparent, the most careful and detailed systematic study of the subfamily. It will be sufficient for the present if the quite natural character of Theobald's genera, taken as a whole, is emphasized. What seems mostly now required is accurate and detailed descriptions of species, with a really comprehensive and authoritative revision of the Anophelinae. But a list of the groups as defined by structural characters and colour markings, and a tentative arrangement of these as they appear to be phylogenetically related, should not be open to any objection. Retaining Theobald's nomenclature, but noting the necessity of a number of emendations as regards the species at present contained in these genera, and subdividing the genera where necessary, the following represents, as far as one can judge, the phylogenetic relationship of these groups.

Towards elaboration of ornamentation and scale development

TENTATIVE PHYLOGENETIC ARRANGEMENT OF THE ANOPHELINAE

In this arrangement, phylogenetic advance is associated with both an increase in colour markings (i.e., the development of white) and in scaling. It is not difficult to arrange a correlation table in which increasing degrees of whitening and increased degrees of scaling form the ordinates and abscissae respectively. The striking nature of the correlation between the two series of characters is then made very apparent by the grouping of species about the diagonal line. The same correlation is also very evident in the phylogenetic scheme given.

Considering the fact that the Culicinae are all scaly, one might think it probable that Anophelines were a branch in which scales were disappearing. But my own observations on this point make it more probable that it is the other way. The more scaly an Anopheline is the more advanced phylogenetically it would appear to be.

Certain of the groups, it will be seen, occupy a much more primitive position in the scheme than others, and as this seems to be demonstrated by whatever character they are considered, and as there are definite characters by which one can distinguish these apparently old type species, I have named them *Protoanopheles*. The peculiar Australasian type I have called *Neoanopheles*. The ordinary Anophelines exhibiting the regular colour scheme I have outlined may approximately be termed the *Deuteroanopheles*. The nature of these groups, and the characters on which they are based, can be obtained from the large table accompanying this paper. Briefly stated, the constitution of the main groups noted is as follows:—

PROTOANOPHELES.

Stethomyia.
Anopheles.
Patagiamyia and *Lophoschelomyia*.
Myzorhynchus, *Cycloleppteron*, *Arribalzagia*, etc.

DEUTEROANOPHELES.

Myzomyia. } Branch A.
Pyretophorus. }
Pseudomyzomyia, etc. Branch B.
Nyssorhynchus. }
Neocellia. } Branch C.
Cellia. }

NEOANOPHELES.

Many spotted winged species and their related types.

Between branches A and C of the Deuteroanopheles there are distinct differences in ornamentation, such as, for example, a much greater tendency to a completely broken third costal spot and narrow costal interruptions, but the most striking difference is in the much greater intensity of the black and white ornamentation. The origin of the branch B is difficult to determine. It is especially interesting as including some of the most dominant species, i.e., *Pm. rossi*, *P. costalis*, *Ne. stephensi*, *Pm. ludlowi*.

GEOGRAPHICAL DISTRIBUTION

Having on structural grounds arrived at certain groupings, it is interesting to note the geographical distribution of these groups.

Stethomyia, though it contains very few species, has a distribution area which includes South America and Australia, as well as India and Malay.

Myzorhynchus, if we consider the closely-related genus *Cyclo-leppteron*, also has a very extended distribution, which includes South America and Australia.

Anopheles and *Patagiamyia* are the dominant genera of Europe and North Amerca. There is, however, what appears to be an Australian representative of this branch, as well as hill species in the East.

In the case of the Deuteroanopheles, certain of the genera have a much more restricted distribution. Thus *Pyretophorus*, in the restricted sense in which I have been using the term, is dominant in Africa (North), occurs in South Europe and in the extreme North-west of India. It does not occur in South America or Australia, and it is scarcely represented in the Anopheline fauna of India and Malay.

Myzomyia is essentially African, Indian and Malayan.

Nyssorhynchus is also African and Malayan, with a distinct dominance in the latter area and in India.

The most widely distributed of the genera of the Deutero-

anopheles is *Cellia*, and this fact is not out of keeping with the position assigned to this genus in the scheme of phylogeny.

The *Neoanopheles* are distinctly Australasian and Malayan.

More than the above outline of the main features of geographical distribution of species cannot be attempted here, but it will be seen that to a large degree consideration of the geographical distribution of species gives distinct support to the ideas respecting phylogeny which I have put forward in this paper.

VII. GENERAL CONCLUSIONS

Colour markings, equally with structural characters, can be utilised in natural classification of the Anophelinae and in the placing of species in groups to which they have affinities. A classification based on colour marking, supported by structural differences in the palps and larval characters, approximates very closely in regard to the groups formed with one based on scale structure. It shows, however, the relation and affinities between the groups much more clearly than does scale structure classification as at present employed in the distinction of genera.

Colour markings, and general characters as a whole, seem to point to there being a group of more primitive forms (PROTOANOPHELES) occurring as old world species (*Anopheles*, *Myzorhynchus* and *Patagiamyia*), and as new world representatives of this group *Cycloleppteron*, *Arribalzagia* and *Myzorhynchella*. There is also a distinct group corresponding to Donitz's Australasian species with more than three spots on the sixth vein (NEOANOPHELES). Their area of special prevalence is Australasia and Malaya.

The majority of Anophelines belong to a group the colour markings of which are all on a given colour scheme (DEUTEROANOPHELES). This group shows two main divisions, characterised by a notable difference in the intensity of their coloration and the 'effectiveness' of ornamentation, possibly arising from two different lines of evolution. Their area of special prevalence is an area including Africa, South Asia and Malay.

I am very greatly indebted to Prof. R. Newstead and to Mr. H. F. Carter for their great kindness in furnishing me with

material for studying a number of African and South American species, including specimens of *Cy. grabhamii*, without which I could not have carried out many of my observations. I am also indebted to Prof. Newstead and Mr. Carter, as well as to Mr. F. W. Edwards, for their kindness in giving me every facility to make use of the collections at Liverpool and the British Museum respectively.

I also wish to acknowledge my indebtedness to Prof. G. H. F. Nuttall and to Mr. H. Scott, at Cambridge, for help in regard to literature and in other respects.

VIII. TABLE SHEWING GROUPING OF SPECIES OF ANOPHELINAE ACCORDING TO COLOUR MARKINGS AND OTHER VARIABLE CHARACTERS

A. NODAL CENTRES AT CROSS VEINS AND BIFURCATIONS DARK. (PROTOANOPHELES.)

A′. Costa devoid of any light areas even at apex. Wings usually without any pale markings.

1. Palps markedly heterodactylous with marked relative elongation of the second segment. Attitude *culex*-like. Wings entirely unspotted. Palps unbanded. Legs entirely without markings even knee spots being absent. Small mosquitos.

(Larval antenna without branched hair. Palmate leaflets lanceolate.)

Head scales linear. No scales on prothorax, mesothorax or abdomen.

Corresponds exactly with *Stethomyia*, Theo.; *Neostethopheles*. James.

S. nimba, Theo.

S. aitkeni, James and Liston.

S. culiciformis, James and Liston.

S. corethroides, Theo.

Probably synonyms—*S. treacheri*, Leicester; *S. fragilis*, Theo.; *S. pallida*, Ludlow.

2. Palps orthodactylous. Attitude *anopheles*-like. Palps thin, unbanded. Primitive spotting may be present. No admixture of light and dark scales. Knee spots usually present otherwise legs are quite unornamented.

(Larval antenna with branched hair (small), palm-leaflets lanceolate and serrated).

Head scales expanded. No prothoracic tuft. No scales on mesothorax or abdomen.

Corresponds with *Anopheles* sense of James.

A. maculipennis, Meigen.

A. bifurcatus, Linn.

A. nigripes, Staeger.

A. algeriensis, Theo.

A. barianensis, James.

A. barberi, Coq.

A. immaculatus, James. (?).

A. eiseni, Coq. (?).

A. crucians, Wied. (?).

A″. Costa with at least one pale interruption which may be at the apex.

(*b′*) *Wings without prominent admixture of pale and dark scales.*

3. Costa dark broken only at apex or at subcosta with or without small basal interruption. Palps usually unbanded. Primitive spotting prominent. Tarsus unbanded.

(Larva with branched hair on antenna. Palmate leaflets with filament but showing approach to lanceolate type.)

Head scales expanded. Tuft on prothorax. No broad scales on mesothorax. May or may not be scales on last segments of abdomen.

3*a*. With white band on femur which may be associated with tufts of outstanding scales in this situation.

Lophoscbelomyia.
L. asiatica, Leicester.
L. lindesayi, Giles.
L. wellingtonianus, Alcock.

3*b*. Without white band or femur.
L. (?) *atratipes*, Skuse.

4. Costa prominently spotted. (Usually two, not more than three, main spots). Palps banded. Primitive spotting may be present (but not conspicuous). White spots on wings for most part represented on lower surface. Large mosquitos.

(Larval antenna carries a branched hair. Palmate leaflets with filament.)

Head scales expanded. Tuft on prothorax. No scales on mesothorax (or abdomen).

Corresponds to *Patagiamyia*, James.

Pt. gigas, Giles.
Pt. simlensis, James.
Pt. punctipennis, Say.
Pt. smithi, Theo. (Unspotted wings.)

Probably
A. formosus, Ludlow.

Perhaps
A. franciscanus, McCracken.
A. perplexans, Ludlow.
A. pseudopunctipennis, Theo.

Would probably include also
Feltinella pallidopalpi, Theo.

5. Costa dark with narrow interruptions but with four main costal spots. Wings markedly dark beneath. Palps shaggy.

(Larval antenna without branched hair. Palmate leaflets lanceolate.)

Head scales flattish. Broad scales on mesothorax. No scales on abdomen.

Myzorhynchella.

(*b''*) *Wings with prominent admixture of dark scales. Pale spots on the wing deficient as a rule on under surface. Hence, when third spot is light it shows dark border of projecting dark scales seen through wing membrane. Primitive spotting the rule. Sixth vein with at least one scale cluster of dark scales.*

(*Larval antennae with branched hair. Palmate leaflets lanceolate and serrated. Frontal hairs markedly branched.*)

Prothoracic lobes with tuft.

6. Legs not speckled. Costal edge dark with two (at most three) minute pale interruptions. Basal portion of costa unbroken by pale areas. Palps markedly orthodactylous, shaggy, unbanded or with scheme A ornamentation. Large mosquitos Old World species.

No broad scales on mesothorax. Some ordinary scales present or absent on abdomen. A ventral tuft of dark scales usually present on penultimate segment of abdomen.

Corresponds to *Myzorbynchus.*

My. barbirostris, Van der Wulp.
My. pseudobarbirostris, Ludlow.
My. bancroftii, Giles.
My. umbrosus, Theo.
My. strachani, Theo.
My. sinensis, Wied.
My. pseudopictus, Grassi.
My. paludis, Theo.
My. mauritianus, Grandpre.

7. Legs markedly speckled. Costal edge may be dark as in last group or broken into distinct spots. Accessory spot often present. Palps shaggy with scheme A ornamentation. Large mosquitos. New World species.

2*a*. Sixth vein with two dark spots. Wing with inflated scales but resembling that of Myzorhynchus in general arrangement.

No broad scales on mesothorax.

Cycloleppteron
Cycloleppteron grabhamii, Theo.

2*b*. Sixth vein with many spots.

Broad scales on mesothorax. Abdomen with many scales and lateral tufts.

Arribalzagia.
Ar. maculipes, Theo.
Cy. mediopunctatus, Theo.
(*Ar. pseudomaculipes*).
(*Ar. malefactor*).
(*Cy. intermedium*).

B. NODAL CENTRES AT CROSS VEINS AND BIFURCATIONS PALE. COSTA WITH FOUR DISTINCTLY MARKED MAIN COSTAL SPOTS.

B′. Palps Ornamented on Scheme A—C. Costa without Accessory Spots other than the basal ones. Not more than three dark spots on Sixth Longitudinal Vein. (DEUTEROANOPHELES.)

(*a*) Tips of hind tarsi not white.

a′. Palps markedly heterodactylous. Tips of hind tarsi never white. Banding of front tarsal joints never broad. Legs never markedly speckled. Wing markings of species on fixed scheme (Scheme A). If three spots are present on sixth vein there is a pale interruption on upper branch of second longitudinal. Completely broken third costal spot unusual.

(*Larval antenna without branched hair. Palmate leaflets with filament. Frontal hairs usually simple unbranched.*)

No prothoracic tuft. No scales on abdomen, even in male.

1. Sixth vein with two or less dark areas. No pale internodal spot on branch of fifth. Branches of second long, rarely with pale interruption. Still more rarely interruptions on branches of fourth. Fifth vein dark at junction of branch.

   Mesothorax without broad scales.

   Corresponds to *Myzomyia* in restricted sense (i.e., not including *Pm. rossi*, *M. lutzi*, etc.).

   M. culicifacies, Giles.
   M. nili, Theo.
   M. rhodesiensis, Theo.
   M. bebes, Donitz.
   M. umbrosa, Theo.
   M. listoni, Liston.
   M. funesta, Giles.
   P. sergentii, according to wing.

2. Sixth vein with three dark spots or two. Pale internodal spot on branch of fifth. Branches of two and four veins with pale interruptions. Fifth vein usually pale at junction of branch. Palps peculiarly long and thin. Not infrequently show apical dark band (four palpal bands) or apex dark.

   Broad scales on Mesothorax.

   Pyretophorus I (*superpictus* group).

   P. superpictus, Grassi.
   P. nursei, Theo.
   P. nigrifasciatus, Theo.
   P. cleopatrae, Willcocks (MSS.)
   P. cardamatisi, Newstead and Carter.
   P. distinctus, Newstead and Carter. Costa unusual but wing otherwise showing typical *Pyretophorus* condition.
   P. palestinensis, Theo.
   P. cinereus, Theo.

a''. Palps not markedly heterodactylous.

3. Upper branch of second vein with pale interruption.

Tip of hind tarsus not white. Palps destinctly orthodactylous but with whole of apical segment pale. Banding of front tarsus broad. Apical segment of palps in spite of length pale throughout. Hind tarsi not white but speckling usual. Whitening of wing considerable with completely broken third costal spot usual. All internodal pale spots developed and some residual pigment areas usually obliterated.

A composite group according to scale structure.

Prothoracic tuft. Preapical costal spot broken. Scales on mesothorax. A few scales on last segment of abdomen especially in male.

Pyretophorus II.

P. costalis, Loew.

(*P. pseudocostalis*, Theo. ?).

(*P. merus*, Donitz ?).

(*P. marshallii*, Theo. ?).

No prothoracic tuft. Preapical costal spot not broken. A few broad scales on mesothorax. A few scales on last abdominal segment especially in male.

Pseudomyzomyia, Theo. = *Nyssomyzomyia*, James.

Pm. rossi, Giles.

Pm. indefinata, Ludlow.

Pm. ludlowi, Theo.

No prothoracic tuft. Nyssorhynchus-like scales on thorax. *Ne. stephensi.*

4. Upper branch of second without pale interruption. Palps orthodactylous and with scheme A pattern. Bridging of pale spots on costa by dark areas on first long vein marked feature.

Prothoracic tuft. Broad scales on mesothorax. Abdomen with lateral tufts.

Cellia squamosa, Theo. Related to *Myzorhynchus* and Cellia. A peculiar species.

Isolated position in this table due to fact it does not possess white tips to tarsus. Note prothoracic tuft.

(*b*) Tips of hind tarsi white. Ornamentation very black and white. Speckling and banding usual. Full development of internodal pale areas of wing, but delayed appearance of pale area on upper branch of second vein.

(Larval antenna without branched hair. Palmate leaflets with filament. Frontal hairs often show some degree of branching.)

Characteristic broad white creamy scales on mesothorax.

5. Palps ordinarily three banded (scheme B). Orthodactylous or with some tendency in light forms to be slightly heterodactylous (*N. maculatus*).

Abdomen with scales on last few segments (*Nyssorbynchus*) or on many segments (*Neocellia*) but without lateral tufts. No prothoracic tuft.

Dark group. Upper branch of second long vein without interruption. Hind tarsi usually show uninterrupted white area extending over several segments. Palps destinctly orthodactylous, ornamented scheme B, with tendency in individuals to revert to A.

N. fuliginosus, Giles.
N. nivipes, Theo.
(*N. freerae*, Banks.)
(*N. phillipinensis*, Ludlow.)
Ne. fowleri, Christophers.
N. jamesi, Theo.
N. pretoriensis, Theo.
N. maculipalpis, James and Liston.

Light group. Upper branch of second long vein with interruption. Hind tarsi usually with alternate black and white areas. Palps with shorter terminal segment than last group and with scheme C ornamentation (two broad apical bands).

N. maculatus, Theo.
N. theobaldi, Giles.
Ne. willmori, James.

6. Palps ordinarily four banded (scheme A) and markedly orthodactylous.

Abdomen with lateral tufts. Prothoracic tuft in some species.

Cellia. Dark group. Bridging of pale spots on costa common. Also absence of interruption on upper branch second.

C. argyrotarsis, Desvoidy.
C. albimana, Wied.
C. jacobi, Hill and Haydon.
C. cincta, Newstead and Carter.
(*C. squamosa*, Theo. Hind legs not white, vide above.)
(*C. squamosa, var. arnoldi*. Hind legs not white, vide above.)

Light group. Upper branch of second interrupted. Costal spots not bridged.

C. pulcherrima, Theo.
C. pharoensis, Theo.

B″. Palps with Scheme D ornamentation. More than three dark spots on the Sixth Vein. Accessory costal spots often present in addition to basal ones. (NEOANOPHELES.)

With prothoracic tuft.

My. natalensis, Hill and Haydon. *
P. watsonii, Leicester. *
Nm. elegans, James. *
(*Nm. leucosphyrus*, Donitz.) *
N. annulipes, Walker. *
N. masteri, Skuse. *
Ch. kochi, Donitz.

Query any prothoracic tuft.

M. punctulata, Donitz. *
N. deceptor, Donitz. *
N. thorntonii, Ludlow.*
N. karwari, James and Liston.

All species show same character of palpal ornamentation.

* Shows more than three dark spots on sixth.

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EXPLANATION OF PLATES

Plate V

Fig. 1. Nymphal trumpets of *A. bifurcatus*, Linn.

Fig. 2. Nymphal trumpet of *Ne. willmori*, James.

Fig. 3. Leaflet of palmate hair of *My. sinensis*, Wied.

Fig. 4. Leaflets of palmate hairs. (*a*) *C. squamosa var. arnoldi*, after Newstead and Carter. (*b*) *N. annulipes*, Walker, after Theobald. (*c*) *A. bifurcatus*. (*d*) *Cyclolepteron grabhamii*, Theo., after Theobald. (*e*) *Myzorhynchus* (?) *natalensis*, Hill and Haydon, after these authors. (*f*) *P. ardensis*, Theo., after Hill and Haydon.

Fig. 5. Antenna of larva. (*a*) *A. bifurcatus*. (*b*) *A. punctipennis*, Say., after Smith.

Fig. 7. Female palp of *My. barbirostris*, Van der Wulp, showing orthodactylous character and unbanded condition.

Fig. 8. Female palp of *C. pulcherrima*, Theo. Orthodactylous with scheme A ornamentation.

Fig. 9. Female palp of *Pt. lindesayi*, Giles. Orthodactylous and unbanded.

Fig. 10. Female palp of *P. nursei*, Theo. Heterodactylous with appressed scales and scheme A ornamentation.

Fig. 11. Female palp of *P. jeyporensis*, James. Heterodactylous with appressed scales and ordinary Deuteroanopheles type of ornamentation, i.e., scheme B, with involvement of the whole of the apical segment.

Fig. 12. Female palp of *P. costalis*, Loew. Orthodactylous with scheme B ornamentation.

Fig. 13. Female palp of *M. funesta*, Giles. Heterodactylous with appressed scales and scheme B ornamentation.

Fig. 14. Female palp of *N. maculatus*, Theo. Scheme C ornamentation.

Fig. 15. Female palp of *N. maculatus* contrasted with palps ornamented on scheme D. (*a*) *N. maculatus*. (*b*) *M. punctulata*, Donitz. (*c*) *Ch. kochi*, Donitz. (*d*) *N. karwari*, James. After James.

Fig. 16. Sketch of palp of *S. corethroides*, Theo., showing remarkable modification in the relative lengths of the different segments. Heterodactylous. Type 2.

Fig. 17. Palp of *S. nimba*, Theo., after Theobald.

Plate VI

Fig. 1. Diagram showing structural features of wing and leucogenetic centres. Nodal points are shown by small circles, internodal by larger ones. Pigment centres are shown dark.

Pigment centres.

Ac'., *Ac''.* Basal accessory pigment areas.
i.m.c. Inner main costal spot centre.
m.m.c. Middle main costal spot centre.
s.a.m.c. Subapical main costal spot centre.
a.m.c. Apical main costal spot centre.
2r., *2a.* Basal and apical centres of stem of second vein.
2'b., *2'a.* Inner and outer centres of upper branch of second longitudinal vein.
2''a., *2''b.*, *2''c.* Outer, inner and middle centres of lower branch of second vein.
3a., *3b.* Residual pigment centres of third longitudinal.
4r., *4a.* Centres on stem of fourth longitudinal.
4'a., *4'b.*, *4''a.*, *4''b.* Centres on fork of fourth longitudinal.
5'a., *5'b.*, *5'c.* Centres on branch of fifth longitudinal.
5r., *5''a.* Characteristic centres of fifth longitudinal vein.
6r, *6m*, *6a.* Centres for sixth vein. *6r.* is Donitz's root spot of sixth.

Nodal points.

h. Humeral.
Sc. Subcostal.
Ap. Apical.
S. Sector.
S'. Accessory sector.

C_1, C_2, C_3, C_4, C_5. Nodal points in connection with cross veins.
C.1'. (?) Nodal point concerned in the formation of the inner pale spot on costa.
C.1''. (?) Nodal point concerned with subapical spot.
1, 2', 2'', 3, 4', 4'', 5', 5'', 6. Nodal points on wing margin.

Internodal points.

2^1, 2^2, 2^3. Internodal points on branches of second vein. The relatively late appearance of 2^1 is characteristic of *Nyssorhynchus* and dark *Cellias*.

3, 3. One or two internodal points of third vein.

4, 4^1, 4^2. Internodal points of stem and branches of fourth vein.

5^1, 5^2, 5^3. Points on main vein and branch of fifth vein.

6^1, 6^2. Points on sixth vein.

Fig. 2. Portion of the wing of a species of *tipulidae*, showing venation homologies and extra cross veins.

h. Humeral cross vein.

C.1′. Cross vein joining subcosta to first longitudinal.

C.1″. Ditto upper branch of second to first longitudinal.

R_1, R_2, R_3, R_{4+5}, Radial system.

R.S. Radio-sector.

S. As in figure 1.

S′. Ditto.

Fig. 3. Showing area of cross veins, and relation to nodal point *S*.

Cv_2, Cv_3, Cv_4, Cv_5. Cross veins 2-5.

S. = Sector, *S*′. = Accessory sector nodal point.

sc. = Subcosta.

Fig. 4. Wing of a species of *Chironomus*, showing pale spots on wing membrane involving the hair-like scales covering the wing and aggregation of hair-like scales to form dark spots at the area of the cross veins.

Fig. 5 to Fig. 13. Diagrams of representative wings, showing arrangement of nodal and internodal points as developed in different groups.

Dark spots represent nodal points which remain pigment areas. Circles half dark represent pale spots not developed on under surface of the wing. Small black dots in circles mean admixture of dark and light scales present. Dark stars mean scale aggregations.

Plate VII

Fig. 1. Wing of *A. maculipennis*, showing primitive spotting.

Fig. 2. Wing of *My. umbrosus*, showing primitive spotting, want of representation of pale spots on under surface of wing, and admixture of dark and light scales. A scale cluster on the sixth vein is also shown.
2a. Under surface.

Fig. 3. Wing of *M. funesta var.*, showing first appearance of nodal points with few internodal centres. Pale scaling not intensely white.

Fig. 4. Wing of *N. maculatus*, showing extreme development of nodal and internodal centres, with residual pigment areas well displayed. White scaling intensely white.

Plate VIII

Diagrams of the spotting of the wings in a number of species of Anophelinae, especially to show arrangement of costal spots. Figs. 1-38.

Fig. 39. Halter of *Cy. mediopunctata*, showing resemblance to unexpanded wing, with light scaling above and intensely dark scaling beneath.

1. *Patagiamyia lindesayi*, Giles.
2. *My. wellingtonianus*, Alcock.
3. *Lophoschelomyia asiatica*, Leicester.
4. *Patagiamyia gigas*, Giles.
5. *Patagiamyia simlensis*, James, *var.*
6. *Myzorhynchus umbrosus*, Theo.
7. Ditto under surface.
8. *Myzorhynchus mauritianus*, Grandpré.
9. *Myzorhynchus sinensis*, Wied.
10. *Cycloleppteron grabhamii*, Theo.
11. Ditto under surface.
12. *Cycloleppteron mediopunctatus*, Theo.
13. *Arribalzagia maculipes*, Theo.
14. *Myzorhynchella nigra*, Theo.
15. Ditto under surface.
16. *Cellia squamosa*, Theo.
17. *Cellia squamosa*, Theo.
18. *Nyssorhynchus fuliginosus*, Giles.
19. *Myzorhynchus* (?) *natalensis*, Hill and Haydon.
20. *Pyretophorus* (?) *watsonii*, Leicester.
21. *Neomyzomyia leucosphyrus*, Dönitz.
22. *Myzomyia* (?) *punctulata*, Dönitz.
23. *Christophersia kochi*, Dönitz.
24. *Nyssorhynchus* (?) *annulipes*, Walk. (Costa only shown.)
25. *Pyretophorus costalis*, Loew.
26. *Cellia jacobi*, Hill and Haydon.
27. *Myzomyia culicifacies*, Giles.
28. *Myzomyia listoni*, Liston.
29. *Pyretophorus cinereus*, Theo.
30. *Pyretophorus nursei*, Theo.
31. *Pyretophorus cardamitisi*, Newstead and Carter.
32. *Pyretophorus chaudoyei*, Theo.
33. *Pyretophorus jeyporensis*, James.
34. *Nyssorhynchus theobaldi*, Giles.
35. *Neocellia stephensi*, Liston.
36. *Pseudomyzomyia rossi*, Giles.
37. *Pyretophorus ardensis*, Theo.
38. *Pyretophorus* (?) *atratipes*, Skuse.

A STUDY OF THE POSTERIOR NUCLEAR FORMS OF *TRYPANOSOMA RHODESIENSE* (STEPHENS AND FANTHAM) IN RATS

BY

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Trypanosomes, obtained from a human source, presenting a posterior position of the macronucleus were first described by Stephens and Fantham.[1] Upon this morphological peculiarity they founded their species *Trypanosoma rhodesiense*. Since the publication of their paper, a similar condition has been described in trypanosomes from human sources by various observers in different regions. The patient whose strain they described contracted the disease in a portion of Rhodesia in which the absence of *Glossina palpalis* rendered it necessary to seek a different carrier. Stannus and Yorke[2] discovered posterior nuclear forms in a strain taken from a case of human trypanosomiasis in Nyasaland. More recently Stannus[3] has noted the presence of these forms in a strain obtained from a case of human trypanosomiasis from Portuguese East Africa, while the Royal Society's Commission[4] confirms the observation of Stannus and Yorke with regard to the presence of posterior nuclear forms in strains from human trypanosomiasis in Nyasaland. The Commission bears out their conclusion that the trypanosome of the human trypanosome disease of Nyasaland is probably identical with *T. rhodesiense*.

The occurrence of posterior nuclear forms is not, however, confined to strains of human trypanosomiasis. Such forms have also been recorded among animal trypanosomiases, for example in *T. pecaudi* by Wenyon,[5] in a strain of *T. equiperdum* by Yorke and Blacklock,[6] and in a strain of *T. brucei* from Uganda by Blacklock.[7]

THE INCIDENCE OF POSTERIOR NUCLEAR FORMS IN *T. RHODESIENSE*

Stephens and Fantham (loc. cit.) observed that the forms of trypanosome which presented a posterior position of the nucleus were the short and stumpy ones, and that in rats forms having this peculiarity made their appearance in the peripheral blood about the fifth or sixth day of the disease, and increased in number to the seventh or eleventh day, when they formed about 6 % of the parasites present.

SHORT AND STUMPY

The term 'short and stumpy' appears to be used in somewhat different senses by different observers, in relation to trypanosomes. For example, Bruce (loc. cit.) says 'There is no free flagellum in the short and stumpy forms.' In a table attached, however, he shows that the amount of free flagellum in the 'short and stumpy' forms (620 individuals) averages 0·6. Stephens and Fantham (loc. cit.) say of the stumpy forms of *T. rhodesiense* which have a posterior nucleus that there is a well-marked blepharoplast and *a very short free flagellum*. It appears, therefore, to be not quite decided what constitutes a 'short and stumpy' form. Is it a trypanosome which measures less than a given number of microns, e.g., 21; is it a trypanosome which has absolutely no free flagellum; or is it a trypanosome which is not only less than a certain length, but also has absolutely no free flagellum? In the experiments given below the large majority of forms which had a posterior nucleus measured less than 21 μ, but numbers were found which exceeded this length. Again, of the parasites which conformed to the definition as regards shortness, numbers had absolutely no free flagellum, while numbers had a very definite portion of flagellum free. In some cases this portion was of considerable length. It was not impossible, for example, to discover forms which while they measured only 19 μ in length, yet possessed a free portion of flagellum amounting to 4 μ.

One finds thus that when studying the posterior nuclear forms in these experiments, one is not dealing with short forms in the strict sense that they measure in all cases less than 21 μ nor with

stumpy forms in the sense that they possess absolutely no free flagellum. There are overlapping forms which do no come under both categories, in fact there are a few which come under neither, yet present the posterior nucleus. For practical purposes, however, it may be said that the posterior nuclear forms, whether free-flagellated or aflagellar, which exceed the measurement of 'short forms,' were few in number. The experiments detailed below were undertaken in order to determine in a given infection in rats the time of appearance of posterior nuclear forms in the peripheral blood, and the numerical relationship which these forms bear to other forms of trypanosome present from day to day.

METHOD OF CARRYING OUT THE EXPERIMENTS

A guinea-pig infected with *T. rhodesiense* and having numerous parasites in its blood was utilized as the source of infective material.

Four groups (A, B, C, D) of rats, each group containing six rats, were selected, the rats being as nearly as possible of the same weight. The parasites per cubic millimetre in the blood of the guinea-pig were estimated by means of the Thoma Zeiss haemocytometer, and dilutions made in a warmed mixture of 1 % sodium citrate and 0·85 % sodium chloride. The dilutions were made so that the rats of the first two groups received 1,000,000 trypanosomes, the rats of the third group 4,000,000, and the rats of the fourth group 8,000,000. The animals were all inoculated intraperitoneally, the amount of the injection in each case being 0·5 c.c.

INCUBATION AND DURATION

The average incubation period of Group A (1,000,000 trypanosomes) was 4·8 days, the average duration of the disease 13·6 days. Group D (8,000,000 trypanosomes) had an average incubation period of 4·5 days and an average duration of 15·5 days. Table I gives details as to the incubation and duration of the disease in the individuals of each group. From this table it appears that no definite variation in either incubation or duration could be attributed to the relative numbers of trypanosomes injected.

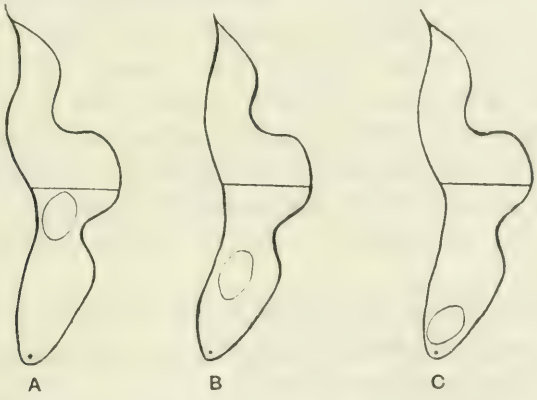
TABLE I.—Giving incubation and duration of infection with *T. rhodesiense* in 24 rats.

Group	No. of Experiment	Incubation in days	Duration
A	Rat 2231 A	5	13
1,000,000	,, B	4	13
Trypanosomes inoculated	,, C	5	14
	,, D	6	14
	.. E	4	14
	.. F	5	14
B	Rat 2232 A	5	13
1,000,000	,, B	6	13
	.. C	5	16
	.. D	5	16
	,, E	4	13
	.. F	6	13
C	Rat 2229 A	5	13
4,000,000	,, B	4	12
	,, C	4	11
	,, D	4	10
	,, E	4	13
	,, F	6	13
D	Rat 2230 A	4	17
8,000,000	,, B	4	17
	,, C	4	15
	,, D	5	14
	,, E	5	15
	.. F	5	15
	Average ...	4·7	13·7

ENUMERATIONS

From the day on which parasites first appeared in the peripheral blood until the death of the animal, a film was examined daily in the fresh state, and a thin film dried and stained. The dried films were fixed in absolute alcohol and stained with Giemsa's stain.

1. *Preliminary count.* In each stained film a count of 200 parasites was made wherever possible. Every trypanosome met with was counted, whether long, intermediate or short, dividing or non-dividing. The number of posterior nuclears was noted, non-dividing forms only being chosen. The result, therefore, gave the number of non-dividing posterior nuclear forms per 200 of all forms. The posterior nuclear forms were classified carefully according to the position of the nucleus. Those forms were classified as A in which the nucleus, although definitely posterior to the centre of the parasite (excluding free flagellum) still lay close to the centre. C forms were those in which the nucleus lay adjacent to the blepharoplast, while B forms were intermediate in position. The drawings (Text-fig. 1) give the positions indicated for each group.



Text-Fig. 1. Showing various positions of nucleus. A. B. C.

RESULT OF THE PRELIMINARY COUNT

The first trypanosomes which appeared in the peripheral blood of each rat were of the long and slender, or intermediate types. Subsequently, short forms made their appearance, and after a few days posterior nuclear forms. In no case was a posterior nucleated

form found in the first day's count of 200 in any rat. The posterior nuclear forms made their appearance in the peripheral blood with considerable regularity, in the following order:— first A forms, next B forms, and lastly C forms. Thus of the twenty-four rats, A forms appeared first in thirteen, B forms first in six. In four, A and B forms appeared together, while in one, A and C forms appeared together. In none of the rats was a C form the first type of posterior nuclear to appear.

DAY ON WHICH POSTERIOR NUCLEAR FORMS FIRST APPEARED

The earliest day of the disease on which posterior nuclear forms were first seen in the peripheral blood in this count was the seventh, the latest on which they first appeared was the thirteenth. The average day of their appearance in the twenty-four rats was the ninth day of the disease. From the time of their first appearing, posterior nuclear forms were usually to be found up to the death of the animal. It was observed that the posterior nuclears increased not only actually as the disease progressed, but also relatively to other forms of parasite. The greatest number of posterior nuclear forms in any 200 counted occurred on the day of death in fourteen out of the twenty-four rats, on the day preceding the day of death in seven rats, two days before the day of death in two rats and previous to this day in the remaining rat.

From the enumeration of the parasites in films from the infected animals by this preliminary count, the results obtained were as follows:—

(1) The first forms of parasite found present in the peripheral blood were long and intermediate free flagellated forms.

(2) Short forms appeared later.

(3) Posterior nucleated forms only appeared after the disease had developed somewhat.

(4) Posterior nucleated forms increased in numbers from the time of their appearance, both actually and relatively to other forms of trypanosome.

(5) Of the posterior nuclear forms those with the nucleus near the centre of the trypanosome appeared first, those with the nucleus

near the blepharoplast last, while the forms with the nucleus between those two extremes made their appearance at an intermediate stage of the disease.

THE CONSTANCY OF APPEARANCE OF POSTERIOR NUCLEAR FORMS IN THESE EXPERIMENTS

Laveran[8] states that the morphological peculiarity of *T. rhodesiense* (posterior nuclears) in rats and mice is not constant. In these experiments, comprising twenty-four rats, there was no exception. Posterior nuclear forms were found in all cases in the preliminary count. Two rats only did not present the advanced form (C form) during this count, but this form was easily found on further search.

2. *Second count.* Two rats out of each group, each having a good infection, were chosen for the purposes of this count. A thousand trypanosomes were enumerated whenever possible in each daily film, and the posterior nuclear forms noted as before. The results of this more laborious procedure confirmed the conclusions arrived at as the result of the first examination, the earliest day on which posterior nuclears were first found being the sixth to the tenth. It appeared also that C forms occurred in greater proportion in those animals which lived the longest time, and that within the group of posterior nuclear forms the C forms generally increased relatively to the A and B forms towards the end of the disease. Table II gives an illustration of some of these points; it gives the number of posterior nuclear forms of the three kinds per thousand of trypanosomes during the course of the disease in two rats.

POST-MORTEM RELATIVE INCREASE OF POSTERIOR NUCLEAR FORMS

In several of the rats from which films were taken after death it was observed that the proportion of posterior nuclear to other forms increased considerably. This phenomenon appears to be related to the observation that frequently after death the 'short stumpy' forms of parasite in *T. rhodesiense* resist the processes of disintegration in the blood of the dead host better than the long forms. Bevan and MacGregor[9] drew attention to this fact. It has also been recorded by Swellengrebel[10] in the mouse, and by Blacklock[11] in the rat.

TABLE II.—To show the dates of appearance of posterior nuclear forms of various grades in the peripheral bood of two rats infected with *T. rhodesiense*, — per 1000 trypanosomes.

Date	Rat 2230 C			Rat 2231 A		
	A	B	C	A	B	C
Oct. 4 ... (Inoculation)	—	—	—	—	—	—
,, 5 ...	—	—	—	—	—	—
,, 6 ...	—	—	—	—	—	—
,, 7 ...	—	—	—	—	—	—
,, 8 ...	—	—	—	—	—	—
,, 9 ...	1	—	—	*—	—	—
,, 10 ...	6	3	—	1	—	—
,, 11 ...	2	2	—	1	—	—
,, 12 ...	*—	—	—	4	2	—
,, 13 ...	1	—	1	—	—	—
,, 14 ...	1	—	2	4	—	—
,, 15 ...	—	1	1	6	2	1
,, 16 ...	4	3	3	10	5	4
,, 17 ...	2	—	1	8	1	1
,, 18 ...	14	12	6	7	1	5
,, 19 ...	6	27	21	—	—	—
,, 19† ...	32	31	22	—	—	—

* Less than 1000 counted on film.

† After death of rat.

THE SIGNIFICANCE OF POSTERIOR NUCLEAR FORMS IN *T. RHODESIENSE*

Many explanations have already been advanced to account for these forms of parasite. Bevan[12] states that the presence of short forms of trypanosome with the macronucleus slightly posterior to the centre is a common feature in many species, and especially in trypanosomes undergoing degeneration or taking on the resistant form in the presence of adverse or unusual conditions. This

explanation does not suffice to explain the typical posterior nuclear forms in *T. rhodesiense*, because in this species it is not a case of the nucleus being *slightly posterior to the centre* merely. There are certainly many forms which conform to this description, but the typical posterior nuclear forms do not fall under this category; they are something much more definite. As regards the suggestion that they are the product of degeneration, it is difficult to explain why they are not found when *T. gambiense* degenerates. As previously stated, there is some evidence that such forms are capable of great resistance to the processes of disintegration in the cadaver. It appears improbable that the same phenomenon, 'posterior nuclears,' could be evidence at one and the same time of degeneration and resistance: it is possible, of course, that the trypanosomes may assume this arrangement of the nucleus in response to the demands of an unsuitable environment, and that subsequently if the environment continues to be unsuitable they degenerate, still retaining this arrangement of the nucleus. But the study of *T. gambiense* under conditions where the parasites are obviously degenerating has not, up to the present, led to the discovery of such forms.

ABERRANT FORMS

Bruce (loc. cit.) refers to the posterior nuclear forms seen by him in the *T. rhodesiense* of Nyasaland as 'aberrant' forms. In view, however, of the fact that they are so constant in their appearance in this strain and form an integral part of it, it seems hardly justifiable to treat them merely as 'aberrant' forms.

DUE TO TECHNIQUE

It has been stated that they may be due to methods of taking films, fixing or staining, but so many observers have noted their appearance, using different methods of treatment for the films, that it seems reasonable to discard this view.

In seeking for an explanation of their presence, it is essential to discover, if possible, whether or not they ever occur in *T. gambiense*. Up to the present, so far as one is aware, they have not been described in this trypanosome. It may be argued

that the number of *T. gambiense* strains which has been examined is small. If, however, it can be established that they are entirely absent from *T. gambiense*, it will be difficult to attribute their presence in *T. rhodesiense* to such changes as degeneration, or to resistance, or to relegate them to the class of 'aberrant' forms. If further examination of strains of *T. gambiense* fails to reveal their presence, it must be concluded that these forms constitute an important distinction between the two parasites *T. gambiense* and *T. rhodesiense*. The fact that such forms are found in animal strains does not diminish their claim to attention, since animals are known to be infected with human trypanosomes. The argument, that if so many animal strains were capable of infecting human beings there would be a great amount of human trypanosomiasis in regions where it does not at present abound, is not conclusive. It has to be definitely proved that there is actually no human trypanosomiasis in such animal-infected regions. The experience in Nyasaland, and in Rhodesia, Northern and Southern, renders it necessary to be cautious in considering a region free from sleeping sickness. Even if human cases were rare, it may fairly be argued that we do not by any means fully understand the factors that govern the successful infection of human beings.

FLY VARIATION

If we regard *T. rhodesiense* simply as a variation of the human parasite of the Gambia, it is possible that the explanation of their occurrence is to be sought, not so much in the conditions of their environment in the blood of human beings and in that of inoculated animals, as in other factors, for example, the transmitting agent, the fly. Against this hypothesis we have the facts that although *T. vivax* and *T. pecorum* are each known to be transmitted by both *G. palpalis*[13] and *G. morsitans*,[14] they do not appear to be modified morphologically by the difference of carrier.

It would be of considerable interest to ascertain whether in cases where *T. gambiense* is transmitted by *G. morsitans* such posterior nuclear forms are to be found in the blood of infected animals.

MULTIPLE INFECTION

The possibility of there being an infection in human beings with more than one species of trypanosome suggests itself. In favour of this one might adduce the facts that not only do animals in nature frequently suffer from mixed infections, but also that certain species of fly have proved themselves capable of transmitting more than one species of trypanosome. It seems probable that human beings are susceptible to mixed infection. The proof and demonstration of a double infection, given that the human being harboured species which were each universally pathogenic to laboratory animals would, with the means at present at our disposal, be practically impossible. In this connection one might refer to the original Gambian Horse Trypanosome described by Dutton and Todd[15]; in this case infection was considered to be caused by a single species of trypanosome, a view which is not now generally accepted. It is only where the pathogenicity of the trypanosomes taking part in a mixed infection is widely divergent as regards laboratory animals that proof of the presence of more than one sepcies of parasite can be given.

Against the hypothesis of mixed infection in the case of *T. rhodesiense* is the evidence derived from transmission experiments. Kinghorn and Yorke,[16] transmitting *T. rhodesiense* by means of *Glossina morsitans* bred in the laboratory, found posterior nuclear forms in all animals which became infected. This implies, accepting for a moment the mixed infection hypothesis, that the flies which became infected acquired and passed on the mixed infection in each case. This is, perhaps, the strongest argument against the hypothesis. A further argument against the possibility is somewhat of the same order, namely, the results of infection by culture of *T. rhodesiense*, which Bayon[17] records.

CONCLUSIONS

1. Posterior nuclear forms first appear in the blood of rats infected with *T. rhodesiense* from the sixth to the tenth day of the disease, taking a count of a thousand trypanosomes.
2. They increase in numbers in the later stage of the disease.
3. They increase relatively to other forms of trypanosome.

4. They cannot be explained as the result of either faulty technique or degeneration.

5. They show definite powers of resistance to disintegration in the cadaver of the animal host.

6. The presence of posterior nuclear forms may be due to:—

(*a*) The occurrence of such forms as a constant constituent of certain strains.

(*b*) A mixed infection.

(*c*) Certain unexplained influences in the blood environment, affecting the parasites.

(*d*) The transmitting agent.

7. There are, at present, stronger arguments against the last three explanations of their presence than against the first.

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ANIMAL TRYPANOSOMIASIS IN THE LADO (WESTERN MONGALLA) AND NOTES ON TSETSE FLY TRAPS AND ON AN ALLEGED IMMUNE BREED OF CATTLE IN SOUTHERN KORDOFAN

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PLATES IX, X

When, some years ago, the Sudan Government took over the region known as the Lado Enclave, which now stretches from the sixth parallel of North Latitude southward to Lake Albert and extends from the Bahr-el-Jebel on the east some 120 miles inland to the frontier of the Belgian Congo, it found itself in possession of an area infected with sleeping sickness. Since that time a great deal has been done by Captain Thompson and Lieut. Ranken, R.A.M.C., in studying this disease and to combat it both prophylactically and therapeutically, but, so far, little attention has been paid to the forms of animal trypanosomiasis present in this part of Africa. The following short and, of necessity, incomplete paper is intended as a preliminary contribution to the subject, and also contains notes on certain other matters intimately associated either with human or animal trypanosomiasis.

I. In May of last year I visited the sleeping sickness camp at Yei, in what is now known as Western Mongalla, the name Lado having been abandoned, as the old Belgian station of Lado on the Nile has disappeared. On the way thither, and when at Yei, I had a few opportunities of examining the blood of transport and other domestic animals. At Loka, a post intermediate between Rejaf and Yei, I found mules infected with trypanosomiasis, and at Yei found a donkey which had been infected when in the fly region of the Moru district further to the north and west.

Lately Captain R. J. C. Thompson, R.A.M.C., who has done such good work in charge of the sleeping sickness camp and in connection with clearance operations, sent me the blood of three of the transport bulls working on the old Belgian automobile road which runs from Rejaf on the Nile, through Loka and Yei, to Aba on the Congo frontier.

He had found these animals infected, and was anxious to know the type of trypanosome present. We will, therefore, first consider the infection in the case of these bulls. A mere glance at the films showed that we were dealing with a trypanosome of the *pecorum-nanum-congolense* group. The films had been fixed in absolute alcohol, and, though rather old, stained sufficiently well with Giemsa to permit of their being accurately drawn at a magnification of 2,000 diameters by the aid of Greil's projection apparatus. This, combined with Bruce's method of measuring, is probably the most accurate means of determining the dimensions of stained trypanosomes. The best results are, however, obtained by fixation with osmic acid. From the three slides 140 parasites were drawn and measured. Attached is the curve (Chart I) obtained on plotting the measurements according to Bruce. It will be found to answer very closely to that for the Sudan *T. nanum*, shown in Vol. A of the Fourth Report of these Laboratories (1911, p. 55). The trypanosome is small, short, and shows no free flagellum. The following statement gives the figures in respect to length:—

	Minimum	Maximum	Average
Length in microns ...	9·8 ...	17 ...	12·6

These dimensions are quite near enough those given by Bruce, Laveran and Mesnil and other observers for *T. nanum* to permit of its being considered as this trypanosome, so far as length goes. The breadth at the broadest part varied from 1μ to 2μ, which is also in keeping with that of *T. nanum*. The accompanying microphotographs show sufficiently well the general aspect of the parasite. Pl. IX, fig. 1 shows a typical short *T. nanum*-like form; fig. 2 a longer variety with a better marked undulating membrane. Neither shows any free flagellum. The infection was, in none of the three cases, very heavy, but I am informed it is proving fatal to the transport bulls. Captain Thompson also informed me that he thinks it may be associated with trypanosomiasis in a herd of cattle

at Mongalla on the east bank of the Nile. I wired to the Governor as to the locality from which this herd came, but failed to elicit any definite information. Archibald,[1] however, has described *T. pecorum* as occurring in a cow from Bor, which is in the more

CHART I. - Curve of measurements of trypanosome from transport bull.

northerly part of the Mongalla Province and on the east bank of the river. Until animal inoculations were carried out, it was of course impossible to decide accurately the species of trypanosome with which we were dealing. Accordingly, I wired to Captain Thompson, asking him if at all possible to inoculate laboratory and other animals, and to let me know the result. I have now heard from him to the effect that an inoculated dog showed trypanosomes in its blood fourteen days after the injection.* It would, therefore,

* These trypanosomes then disappeared and were not again found, while the dog has remained throughout in good condition. Fifteen rats which were inoculated died within 48 hours of inoculation from some unknown cause. It would seem, therefore, that the bull trypanosome may have been *T. nanum* after all.

seem that we are dealing with *T. pecorum*, not *T. nanum*. Certainly fig. 2 shows a trypanosome more of the *pecorum* than the *nanum* type, and also (if we accept the views of Laveran and Mesnil[2] as to *T. congolense* being a distinct species) than the *congolense* type. As a matter of fact, so far as the measurements go, they are nearer those given for *T. pecorum* than for *T. nanum*. In any case the positive result of animal inoculation puts the matter beyond doubt so far as these two species are concerned. (But see footnote on p. 115.)

If the infection has been derived along the automobile road in Western Mongalla (late Lado Enclave) the vector is in all probability *Glossina palpalis*, as *G. morsitans* is not found in this locality, and the only other biting flies in evidence are *Haematopota*. It is, however, very probable that these bulls were infected before going to Western Mongalla, and that some species of *Tabanus*, *Chrysops*, or *Stomoxys* is the active agent in transmitting this form of animal trypanosomiasis in the Southern Sudan. I have mentioned the donkey from the Moru country. I kept only one film of this animal's blood, and, as the infection is very small, have not troubled to draw and measure the parasites. The trypanosome, however, is undoubtedly of the same type as that found in the bulls (fig. 3). Again, if we accept Laveran's view, I think it will be admitted that it is very like *T. congolense*. Considering the history of this trypanosome, it is exceedingly likely that it is *T. congolense*, but there is, of course, no definite proof so far.

As regards the infected mules at Loka, I took films and fixed them in absolute alcohol, having no osmic acid with me. Later these films were stained with Giemsa. The infection was somewhat sparse, and at a magnification of 2,000 diameters it took a long time to draw even as many as a hundred from different slides. This, however, was done, and they were measured by Bruce's method, the curve being given in Chart II.

The measurements in respect to length are as follows:—

	Minimum	Maximum	Average
Length in microns ...	10·5 ...	28 ...	17·6

Figs. 4 and 5 show the general aspect of the parasites. It will be seen that this trypanosome is of the *brucei* or *gambiense* type

in that there is a long and comparatively slender form with a free flagellum, and a short, stout form with hardly any free flagellum. (Pl. IX, fig. 5, shows a form with the blepharoplast just beginning to divide.) The breadth of the former varies from 1·2μ to 2μ, of the latter from 1·7μ to as much as 5μ, there being a few very stout and swollen-looking forms. These did not seem in any way degenerate, but the appearance they presented was possibly artificial and due to slow fixation permitting of contraction and broadening. The usual breadth of these forms was from 2·5μ to 3μ.

A marked feature of this trypanosome was the frequent occurrence of an unstained area, usually of a spherical shape, in the neighbourhood of the blepharoplast. This is, of course, sometimes

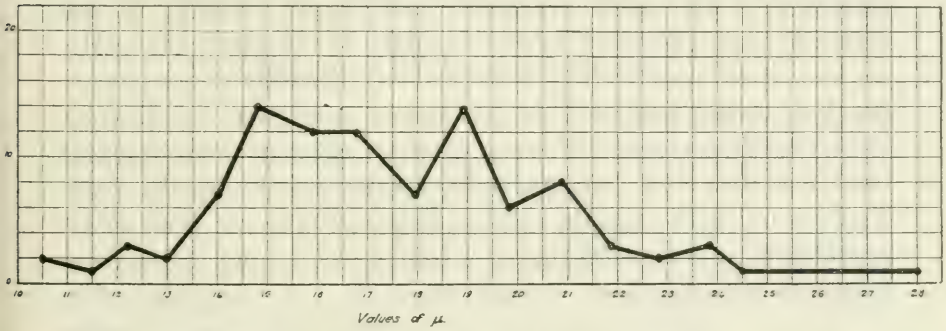

CHART II. Curve of measurements of mule trypanosome.

seen in *T. gambiense* and other species, but I have never known it so frequent or well defined as in this trypanosome. It was specially evident in the short, stout forms, but was occasionally seen in the forms showing a long free flagellum (figs. 4 and 5). Possibly it is merely the product of a faulty fixation. It will be noticed from the curve that a few dwarf or stumpy forms were present. Whether these indicate that there is a double infection, or whether they are like the so-called 'tadpole' forms which have been described in *T. dimorphon* infections, I am unable to say. One of the sick mules accompanied me on my return journey from Loka to Rejaf. At the latter place I had a couple of gerbils on board our floating laboratory. These animals (*G. pygargus*) are as a rule very susceptible to trypanosome inoculation,* but though

* Infection invariably results after inoculation with *T. gambiense*, *T. evansi*, *T. brucei* (*pecaudi*), and *T. pecorum*.

I gave each of them a large dose of citrated blood from the sick mule neither developed any infection.

Under these circumstances, I am unable to express any opinion as to the species which this trypanosome represents. The curve, Chart II, is certainly rather peculiar, there being two apices. It may be that a sufficient number of trypanosomes has not been measured, or, again, it is possible that had the fixation been better the staining of the free flagella might have been better also, and the two apices might then have been compounded into a single apex at or about 19μ. Even in this case, however, it would not have answered very closely to that given by Bruce for the Uganda strain of *T. brucei*. I suggest that it may be the same pleomorphic trypanosome (*Trypanosoma*, sp. ?) found by Kinghorn and Yorke[3] in a bushbuck. The mule transport had been working in the southern Bahr-el-Ghazal before coming to Western Mongalla, so that it is possible the infection was derived in the former province, where but little is yet known regarding the different strains of trypanosome affecting domestic animals. In the past there has been free communication between what was the Lado Enclave and both Uganda and the Belgian Congo, so that, in all probability, various trypanosome infections of bovines and equines have been introduced into Western Mongalla. It is very desirable, as has been shown by the work of Kinghorn and Yorke in many reports, notably those cited,[3,4] as also by Duke[5] and by numerous French observers in West Africa, to determine accurately the different species of trypanosome affecting domestic stock and their insect vectors. These short and incomplete notes may be of some help to those whose duty it will be to work out the problems on the spot, for there is now a good laboratory at Yei, and Lieutenant Ranken intends to extend his researches, both to the disease in domestic animals and to the question of the presence of trypanosomes in big game. The region is so remote and difficult of access that it is well-nigh impossible to carry out the work satisfactorily in Khartoum.

II. In the British Medical Journal for July 6th, 1912, I[6] drew attention to the subject of tsetse fly traps. I have now had large traps constructed by the Andrés Maire Company. These (Pl. X, fig. 6) are not exactly what I intended. Indeed, they differ very

little from the cotton moth trap invented by this firm, but both Mr. King and I think they may serve the purpose of trapping tsetses, provided it is found the flies will enter them.

As regards methods of attraction, the following measures will be tried:—

1. Soaking the central bands or wicks in a mixture of water and either human or animal sweat.
2. Placing a live animal in the trap—a suggestion made by Captain O'Farrell.
3. Soaking the central bands in citrated blood, and at the same time hanging up in the trap a piece of fresh meat from ox or buck with skin still adherent.
4. Soaking the bands as above and having in addition a tube of citrated blood arranged according to the ingenious device of Rodhain and his colleagues.[7] If desired, the blood in this tube may be poisoned.

It is hoped that one or other of these measures may be effective. Lieutenant Ranken will shortly take two of these traps with him to Yei, and carry out the necessary experiments.

III. Fig. 7 is a photograph of a full-grown bull of the small black breed found in Southern Kordofan. It was kindly sent me, at my request, by Major R. S. Wilson, Governor of the Nuba Mountains Province.

This breed is said to be immune to trypanosomiasis, and is the only breed which can live in the infected Koalib area, where *G. morsitans* abounds and conveys an infection due to what I believe is *T. brucei*. It is interesting to note that an apparently similar breed exists in Northern Nigeria. Pollard[8] reports that 'There is in the Munshi Division, and in the northern part of the province, a small black breed of cattle which is apparently immune to tsetse. At any rate, these cattle can be kept in the Munshi district, where no horses can live and where imported Fuhlani cattle all die. I have not yet obtained any blood-films from these animals.'

The Kordofan breed may, of course, only be immune to one species of trypanosome, and, where only one specimen of these cattle is available, it does not seem desirable to make experiments in this

direction. On the other hand, it will be interesting to note the effect of the blood serum of this bull on various trypanosome strains. Some work along this line has been commenced, and will be continued as circumstances permit. At present it is too early to make any statement regarding it.

I am indebted to Mr. G. Hunt, of the Engineering School of the Gordon College, for kindly plotting the curves for me. The photographs are the work of Mr. George Buchanan.

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EXPLANATION OF PLATES

Trypanosomes magnified 2,000 diameters approximately.

PLATE IX

Fig. 1. *Trypanosoma pecorum?* From bull. Note short, stout forms.

Fig. 2. *T. pecorum?* Long form. From bull.

Fig. 3. *T. congolense?* From donkey.

Fig. 4. *Trypanosoma*, sp. ? From mule.

Fig. 5. *Trypanosoma*, sp. ? From mule.

Plate X

Fig. 6. Tsetse fly trap.

Fig. 7. Pigmy bull of black breed from Southern Kordofan, said to be immune to ' fly.'

SANITATION ON THE PANAMA CANAL ZONE, TRINIDAD AND BRITISH GUIANA

BY

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(Being part of the Report on the Malaria Expedition to Panama, 1912)

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PLATES XI, XII, XIII

CONTENTS

	PAGE
INTRODUCTION	125
COMMENCEMENT OF SANITATION ON THE ISTHMUS	128
SANITATION IN COLON	128
SANITATION IN PANAMA TOWN	129
SANITATION ON THE ZONE OF THE CANAL—ANTI-MALARIA WORK	130
ANTI-YELLOW FEVER WORK	136
SANITARY MEASURES ADOPTED TO SAFEGUARD AGAINST PLAGUE	137
MEASURES AGAINST ANKYLOSTOMIASIS	137
ANTI-TYPHOID AND ANTI-DYSENTERY WORK AND GENERAL SANITATION	138
THE HOSPITALS ON THE CANAL ZONE	138
RESULTS OF SANITATION ON THE CANAL ZONE	140
SANITATION IN TRINIDAD	141
SANITATION IN BRITISH GUIANA	143
REFERENCES	146
EXPLANATION OF PLATES	148

INTRODUCTION

In September, 1912, I was sent on an expedition to the Panama Canal Zone by the Liverpool School of Tropical Medicine to study certain malarial problems with Dr. James, Chief Assistant Physician to the Ancon Hospital. The American Canal Commission kindly allowed me to stay in the Ancon Hospital, and it was there that we conducted our researches. Although occupied for the most part with special research, I was nevertheless able, during my three months' stay, to see most of the sanitary work going on. I received much information on the subject from the medical men in the

hospital, and Colonel Gorgas, Chief Sanitary Officer, Colonel Phillips and Mr. Le Prince, Chief Sanitary Inspector, were always willing to give me all the information that I required regarding their organisation and the methods employed. Moreover, I walked over most of the line of the Canal Zone with Dr. Orenstein, Chief Assistant Sanitary Inspector, who was most kind in showing me everything that he possibly could regarding the anti-malaria work. After all that I have seen there, I feel compelled to express much admiration regarding the great system of sanitation, and nothing was apparently left undone in the eradication of malaria and other diseases in general. There was no apparent defect in Colonel Gorgas's sanitary administration. All trusted him in his work with implicit confidence, and the highest authorities gave him power to do what he considered best in great as well as in small measures. The money required was granted with the full belief that it was necessary for the good health of the American inhabitants, and for all workers on the Canal. They realised that good health was to be obtained at all costs, not only for its own sake, but in order to enable them to finish the great undertaking which they had commenced. The Americans, in the Canal Zone at least, are convinced that sanitation 'pays.' They had before them the great lesson of the disastrous failure of the French Canal diggers, and they have learnt that lesson well. No one who had not lived on the Canal Zone before the Americans took it over could possibly realise fully the great changes that have been brought about. The Ancon cemetery, which is a veritable forest of tombstones erected chiefly to French victims (Pl. XII, fig. 9), helps one to realise that in bygone times the risk of death was very high. In an unhealthy tropical country like West Africa, there is always a feeling of insecurity. People are constantly sick, many die, and one feels that perhaps one's own turn is coming next. Mosquitos abound, one is constantly bitten; mosquito nets may be used, quinine may be taken every day, and even though one is careful to drink only filtered or boiled water, yet a feeling of security against malaria and dysentery is never felt. So it was in Panama less than ten years ago. Formerly mosquitos were a plague on board vessels lying in Colon harbour, but since the American occupation that is all changed, and now no mosquitos come aboard. I

arrived on the Canal Zone fully equipped, among other things, with a mosquito net and plenty of quinine which are always so necessary in tropical expeditions, but soon realised that both the mosquito net and the quinine were not needed. There were no mosquitos about, and every American house was mosquito-proof. My three months' stay covered the latter part of the rainy season and the commencement of the dry season, yet during all that time I feel sure that I was not bitten by a single mosquito,* and in consequence I did not take one single grain of quinine. The water supply was good, and was frequently examined bacteriologically. Healthy European women and children were met everywhere, no one seemed to be ill, and one felt as secure from disease as anywhere in temperate climates. I have been in Sierra Leone, Forcados, Bonny and Old Calabar on the West Coast of Africa, also in Trinidad, British Guiana and Jamaica. In all of these places I was bitten by mosquitos, even though my stay had been only for a few days. In all I had to take quinine as a prophylactic against malaria. In these places, moreover, there are not so many white women and children seen as on the Canal Zone at Panama. In West Africa there are no white children and few white women, for the simple reason that it is too unhealthy. I think I have said enough to indicate, without going into the statistics of death-rate, etc., that Panama, once notorious as a white man's grave, is now as healthy as most temperate countries, and the favourable reports which may be read concerning this matter are in no way exaggerated. I have known several traders whose occupation has brought them many times to the Canal Zone during the last ten to fifteen years, and all alike marvel at the great changes which have occurred for the better in so short a time. Most of the authentic reports on the improved health in Panama have been gleaned from the statements made by Colonel Gorgas. He has in no way exaggerated the results of his sanitary work. Again, it must be remembered that this greatest achievement in tropical sanitation, which the world has yet seen, reflects great credit, not only on Colonel Gorgas and his staff, but also on the highest officials on the Canal Zone and on the American Government who have supplied the money without

* Mosquitos are fairly common still at Frijoles and Monte Lirio.

complaint and without putting obstructions in the way, actively or passively, and who have been among the first to realise that thorough sanitation in the tropics 'pays financially.'

COMMENCEMENT OF SANITATION ON THE ISTHMUS

Plans for the sanitation of Colon and Panama formed part of the discussion at the American Commission of 1899-1901. These towns, although situated on the Canal Zone, belong to the Republic of Panama. In January, 1904, the quarantine of Colon and Panama was turned over to the United States, and in June of that year the permanent sanitary organisation was established with Colonel W. C. Gorgas as head and Dr. H. R. Carter, a yellow fever expert, as the Director of Hospitals. The work was hampered by scarcity of supplies, notably copper wire screening, which could not be purchased in the United States in large quantities. An epidemic of yellow fever, lasting from July, 1904, to December, 1905, accelerated the delivery of supplies, and made it necessary to expedite the sanitation work, lest the force slowly organising be depleted. There were 246 cases and 84 deaths, all of which were among the non-immunes who had come to the Isthmus on account of the Canal work. The Americans commenced sanitation, therefore, before attempting any operations on the Canal. They realised that it would be advantageous to make the place healthy before bringing the white population there. They were pioneers in that they built every house mosquito-proof.

SANITATION IN COLON

This town is situated at the Atlantic end of the Canal. It has a population of 18,000. In 1904 it had a population of 10,000, and about 9,000 of these lived in shanties built on piles. At high tide the houses were surrounded with water, so that no one could walk along the streets without danger of falling into the mire (Pl. XI, figs. 1, 3). Behind the town was a large mangrove swamp. There was no proper water supply, and no sewage system. Mosquitos abounded, and malaria and other diseases ran riot. Since then the town site has been filled in by the Americans. It has been raised several feet, so that it is now dry, well paved (fig. 2),

clean, well drained and healthy, with a modern sewage system and water supply. The Panama Republic and the Panama Railway Company are paying for the work.

SANITATION IN PANAMA TOWN

Panama is situated near the Pacific end of the Canal, and has a population of about 37,000. As already stated, there was a yellow fever epidemic raging there from July, 1904, to December, 1905. This, however, was the last of the disease in that town. The Americans in a little over a year eradicated it entirely, and the conditions are now such that it could hardly exist there again. This alone is a triumph which must have already paid financially many fold. Panama Town had previously been an endemic yellow fever centre for centuries, and in a little over one year the disease was stamped out and not a single case has occurred there since. The methods adopted to control the epidemic were isolation of all cases as well as suspected cases, in mosquito-proof quarters, and thorough fumigation of all houses which had cases or suspected cases, in order to kill the mosquitos in them. At the same time a campaign was started to destroy the breeding places of the *Stegomyia fasciata*. At that time every house in Panama had roof gutters and water tanks or barrels, this being almost the sole water-supply of the town. There was no proper drainage or sewage system. The streets were unpaved, without proper gutters, and in the rainy season became a hopeless quagmire (fig. 4). The conditions, therefore, favoured greatly the breeding of the *Stegomyia fasciata*. The Americans employed no makeshift methods in remedying these defects. They brought in a proper pipe-borne water-supply from a reservoir. They installed into every house modern closets, with a pipe-borne sewage system. They paved and guttered every street (fig. 5), and removed as far as possible all the roof gutters from the houses along with the water barrels. They enforced strict sanitary regulations on the inhabitants of the town, and established a health department to see that these regulations were carried out. In addition, the ground floors of the houses were rendered uninhabitable to rats by cementing, and cement gutters were constructed alongside the pave-

ments in order to catch the rain from the gutterless eaves of the houses. These were the drastic measures enforced on the inhabitants of Colon and Panama. The Americans lent the money and supplied the labour. It cost the two towns about £455,000. They are paying it back with interest through water rates which are collected directly by the United States. The inhabitants who had lived there in a slovenly way without interference, as their forebears had done for centuries, were naturally very angry at this apparent tyranny, but already they are becoming satisfied. Those who have families have already noticed an unprecedented freedom from disease among their children. Plague, typhoid fever, dysentery and malaria are no longer to be feared, and now they realise that what was done was good. The sanitation of Colon and Panama is to-day as up-to-date as in any town in Europe. The water-supply is examined very frequently by expert bacteriologists. The quarantine at Panama and Colon is most rigid. Guayaquil in Ecuador is an endemic yellow fever centre, and is only about four days' sail from Panama, hence a very strict quarantine is necessary. In fact, if the Ecuadorians do not soon take steps to make their town sanitary, it is highly probable that the Americans will force them to do it, as it is a constant menace to the health of Panama. Trinidad and all Venezuelan and Colombian ports are under quarantine for yellow fever, though I believe that since my return Trinidad has been removed from the list.

SANITATION ON THE ZONE OF THE CANAL—ANTI-MALARIA WORK

On the Canal Zone the most prevalent disease has been malaria, and much of it is pernicious malaria. The sanitary measures adopted were, therefore, principally anti-mosquito measures. Countless swamps and Anopheles breeding places existed along the line of the Canal. A huge swamp lay behind Colon, and extensive swamps also surrounded the town of Panama. After enormous labour, these have been filled up by material excavated from the Canal. Two methods have been adopted in filling the swamps. One consists in dumping material (rock and earth) into them from railway waggons. The other method, known as a 'hydraulic fill' (Pl. XII, fig. 8), is to force into them a liquid stream of mud

and sand through pipes leading from large suction dredgers employed in deepening the Canal. In many cases these pipes are laid long distances, even a mile or more distant from the Canal. I have seen swamps filled in this way to the depth of many feet, so that only the tops of the trees which grew in these swamps are now visible (fig. 8). Large unhealthy areas have been obliterated in this way. The present town of Balboa on the Pacific entrance to the Canal is being built on one of these filled-in swamps. Strictly speaking, however, these huge undertakings can hardly be considered as anti-malaria work. In describing this, I cannot do better than give an abstract of a paper by Colonel Gorgas (1909) on the subject.

He states that the anti-malaria work on the Isthmian Canal cannot be well understood unless preceded by a brief description of the sanitary organisation as it bears upon this work. The bulk of the activities of the Department of Sanitation on the Isthmus has no immediate bearing upon sanitation, namely, that which deals with religious instruction, care of the sick, care of the insane, of lepers, street cleaning, garbage collection, etc. The Department of Sanitation spent in 1908 some two million dollars, but only five hundred thousand dollars (£100,000) of this was spent on pure sanitation. The American Canal Zone consists of a strip of land ten miles wide, of which the Canal is the centre. This strip extends about forty-five miles in length from North to South. The population to be protected against malaria consists of about 50,000 labourers and their families, and is scattered all over this five hundred square miles, though they are principally collected along the line of the Canal, more particularly into some forty camps and villages near this line. The temperature, rainfall, and character of the terrain are all excellently suited for the breeding of Anophelines all over this territory (fig. 7). The rainfall averages over 100 inches yearly, and though there are four months in which there is practically no rainfall, there is enough water for the Anophelines to breed freely during these four months. During the five years of occupancy of the Isthmus up till 1909, 250,000 people have resided in this zone, and these have been located principally in places formerly unoccupied along the line of the Canal, and as the villages are intended for only temporary

occupancy, the conditions are a good deal like those of an army going into a new country, and increase the difficulty of sanitation enormously.

The anti-malarial measures consist:—

1. In destroying the habitat of the Anophelines, during their larval stage, within a hundred yards of dwellings.

2. Destroying within the same area all protection for the adult mosquito.

3. Screening all habitations so that mosquitos cannot have access.

4. Where breeding places cannot be destroyed by draining, use is made of crude oil and larvicide.

These measures are given in the order in which they are considered important.

For the purpose of carrying into effect these measures, the five hundred square miles of territory have been divided into seventeen districts. These districts are under the charge of a Chief Sanitary Inspector, who has in his office the necessary clerical force and three assistants. One of these assistants is especially competent in the life-history and habits of the mosquito, another in knowledge of ditching, tile-draining, etc., and the third in knowledge of general executive work. Each of the seventeen districts has a District Sanitary Inspector in charge. Each District Inspector has a sufficient force of labourers (forty to fifty) to do the necessary ditching and draining. A force of carpenters is required to keep the screening in repair, and there are one or two quinine dispensers who are kept constantly going around giving doses of quinine to those who desire it. It is not attempted to enforce prophylactic quinine. The Chief Sanitary Assistants are kept constantly going over the work advising and instructing the District Inspectors. The District Physician sends in daily to the central office a report of the number of cases of malaria and the number of the employees from which these cases come. This report is made up weekly in the central office. A copy is sent to each District Inspector, and he is held responsible for any excess of malaria in his district. If the admission rate for malaria during the week rises above $1\frac{1}{2}$ per cent. something is considered wrong, and the assistants of the Chief Sanitary Inspector are sent to look over the

ground to try to discover the cause. The District Inspector, for the purpose of doing away with the breeding places of larvae, puts down tile drains wherever they would be suitable. This is considered the most effective and economical form of drainage. After it is laid down it requires no more attention. There is no breeding place left for mosquitos, as no water whatever is exposed at the surface. A horse mower or scythe can be used for cutting the grass over it. Where tile-drainage cannot be used, open concrete ditches are put down. The first cost of this is nearly as great as that of tiling, and a certain amount of labour is necessary to keep the ditches clear. They must be swept out once a week. If the ground cannot be drained in either of the above ways, open ditches are used. This is the least effective and most expensive form of drainage. In Panama they fill up rapidly with grass, and have to be cleaned out about once in two weeks. They are always breeding places for mosquitos, and have to be constantly treated with oil or larvicide.

For the purpose of doing away with places which will harbour the adult mosquito, the inspector clears the ground of brush and grass for a hundred yards around the place to be protected. Where the locality is to be occupied for a year or more, it is more economical to grade the ground and plant grass, which can then be kept down with a horse mower or scythe. A limited amount of shrubbery and a few trees about a dwelling are not objectionable. The inspector keeps the wire screening in repair by constantly going over it with a force of carpenters. Good wire should last on the Isthmus at least three years. The inspector uses crude petroleum or larvicide in such places as cannot be drained, and in temporary pools caused by construction or at temporary camps where it would not be economical to drain. Usually when a new location is occupied the malaria rate is high, frequently as high as 25 per cent. a week, but always in the course of a month or two, when the ground is drained and the brush cut, this drops to a rate somewhere about 1 per cent. The above methods could be applied to a considerable extent to military organisations. Where troops remained at one camp for a week or longer, it would be practicable to clear and drain the ground. It is most important that the Sanitary Inspector should attend to the work himself. The men

who do the ditching and brush-cutting, etc., should be immediately under his control, and he should be held responsible for the proper performance of the work, or in other words, the work must be constantly supervised by a man who has a considerable knowledge of these anti-malarial measures. Prophylactic quinine is looked upon as an important measure, and is offered in three grain doses to all employees who will take it. It is placed on the table in all the messes in liquid and pill form, and one to three dispensers are employed in each district who go around the various villages.

Fuller details may be had from articles by the following authors:—W. C. Gorgas (1904, 1906, 1907, 1908, 1909, 1910, 1912), A. J. Orenstein (1911, 1912), J. A. Le Prince (1908), and S. T. Darling (1910, 1912); also from the Reports of the Department of Sanitation of the Isthmian Canal Commission by W. C. Gorgas, and from the Canal Record.

The anti-malarial work, therefore, on the Canal Zone consists simply in carrying out thoroughly and in a straightforward manner the anti-malarial principles laid down by Ross soon after his discovery of the transmission of malaria by the Anopheles mosquito. Yet simple though these principles seem, the methods and organisation of Colonel Gorgas deserve the most careful study. His system has produced most remarkable results in a country which was exceedingly unhealthy and in which the difficulties were great and many, and although the construction of the Canal brought unlimited labour and material to his aid, yet it also greatly increased his difficulties, because it is well known that malaria tends to increase greatly where land excavation is going on, on account of the innumerable new breeding pools which are produced by these excavations. This alone necessitates the constant inspection and tackling of ever-changing conditions. The working population is constantly changing its location, and this also involves many difficult sanitary problems and increase of sanitary work. The working population on the Canal moves about so much that these methods are even suitable, as Colonel Gorgas has said, for military organisations in the tropics. It seems to me that the keynote of the success is thoroughness in every detail. With regard to mosquito-proof buildings, even the signal-boxes on the railway are mosquito-proof, and special arrangements have been made so that mosquitos

cannot gain an entry where the wires run into these buildings to the operating levers. The quarters in which the European labourers live are mosquito-proof, but it is found that they are careless in leaving the doors open at times. To safeguard them, mosquito catchers are sent into these dwellings every morning to catch the mosquitos which have gained entry. Most of the insects are filled with blood, having fed during the night. Many mosquitos which might have become infected are therefore destroyed. Dr. Orenstein (1912) has found that this procedure reduces the malaria rate. Oil and larvicide are used so freely that it is very difficult to find any trace of larvae in pools not yet drained or filled in or in ditches not yet concreted. In order to emphasise further the thoroughness of the sanitation on the Canal Zone, I give the following list of the personnel under Colonel Gorgas.

The personnel of the Division of Zone Sanitation is as follows:—

1 Chief Sanitary Inspector.
1 Chief Assistant Sanitary Inspector.
2 Division Inspectors.
26 Inspectors.
1 Inspector—Entomologist.
18 Foremen and 226 labourers.

The following is a summary of the routine work accomplished by this personnel during the month of July, 1912:—

Anopheles work:

Linear feet of ditches cleaned	129,150
Linear feet of new ditches dug	710
Square feet of grass cut and removed	736,300
Number of loads of grass removed to dump	494
Number of cubic yards of earth used in filling holes, etc.	28
Districts oiled, each four times in routine: Chorrillo, Cocoa Grove and Avenida Ancon; San Felipe, Santa Ana and Trinchera; Pueblo Nuevo, San Miguel and Caledonia; Guachapali, Maranon and Panama Railroad yards; special oiling as found necessary.	
Larvacide used .....gallons	500

Disinfection and fumigation:	
Houses disinfected and fumigated for diphtheria...	3
Houses disinfected and fumigated for typhoid fever	1
Houses fumigated for yellow fever.....	6
Number houses disinfected	4
Number houses fumigated	10
Number rooms disinfected	10
Number rooms fumigated	38
Total number cubic feet disinfected	16,300
Total number cubic feet fumigated	369,300
Destruction of rats:	
Rats caught in traps	496
Number of rat traps in use daily	200
Inspection of houses and yards:	
Number of yards cleaned.....	132
Loads of refuse hauled to dump.....	33
Number of old buildings condemned and demolished	1
Number of notices to abate nuisances served by inspectors	42
Number of inspections by reinspector	123
Number of notices complied with	39
Number of letters to alcade requesting enforcement of sanitary rules and regulations	1
New buildings:	
Number of plans submitted to Health Officer and approved	19

The Sanitary Inspectors are thoroughly trained men. They undergo courses of instruction and have to pass examinations. I would refer those interested to the 'Manual of Instruction for Sanitary Inspectors,' published by the I. C. C. Press, Mount Hope, Canal Zone, 1912. Everything goes to show the thoroughness of this great sanitary organisation.

ANTI-YELLOW FEVER WORK

Despite the fact that no case of yellow fever originated on the Canal Zone since May, 1906, yet the efforts to eradicate the *Stegomyia fasciata* have not been abated. The Sanitary Inspectors

exercise great vigilance in preventing accumulations of water in containers where *Stegomyia* might breed. Roof gutters are not allowed, except self-draining, short gutters over the entrance to the house. No water containers are permitted at any house within a 300 foot radius of a public water supply, nor, of course, at any house where there are water connections. In localities more than 300 feet distant from a public water supply, only screened containers are allowed. The Sanitary Department supplies the material for screening one container at each house. Measures for eradicating *Stegomyia* have been so successful that an adult *Stegomyia* is considered a great curiosity, and it is becoming difficult to obtain *Stegomyia* larvae in the sanitated area of the Canal Zone.

SANITARY MEASURES ADOPTED TO SAFEGUARD AGAINST PLAGUE

The quarantine is exceedingly strict, as plague is endemic in Guayaquil, which is only four days' sail from Balboa. Houses are rendered as far as possible rat proof by cementing the floors or by building them on piles. Rats are constantly being trapped and poisoned, and all rats caught or found dead are immediately immersed in antiseptic and sent to the laboratory for examination. Night soil and garbage are placed in special garbage tins with lids. It is removed daily to be burnt and buried. It is the duty of the Sanitary Inspectors to attend to this.

MEASURES AGAINST ANKYLOSTOMIASIS

This disease is very common, especially among the negroes. It is combated by the establishment of a very thorough latrine system along the line of the Canal. The Sanitary Inspectors look after the latrines in their respective districts. The latrines are fly-proof earth closets, and their position is frequently changed. When the contents of the pit reach within two feet of the ground surface, the pit must be filled, after liberally covering the contents with unslaked lime or other disinfectant. The fill must be banked at least 18 inches above the surrounding ground surface. Patients coming into the hospitals and dispensaries are constantly examined for ankylostome ova, and they are given a thorough course of santonin treatment if these are found.

ANTI-TYPHOID AND ANTI-DYSENTERY WORK AND GENERAL SANITATION

The reservoirs and their watersheds are kept free from human habitation and trespass, and the water is periodically examined chemically and bacteriologically.

Garbage cans are cleaned daily, and the garbage is either burnt or buried each day. Manure is burnt to prevent fly breeding. Pit closets are rendered fly proof. Literature is distributed among the people to call attention to the danger of flies.

Sanitary Inspectors carry out all the fumigation and disinfection necessary. In bacterial diseases, such as diphtheria and typhoid fever, thorough disinfection of utensils, floor clothing and bed clothes is carried out.

THE HOSPITALS ON THE CANAL ZONE

There are two principal hospitals on the Canal Zone. One at Colon on the Atlantic side of the Canal, with 450 beds, and the other at Ancon on the Pacific side of the Canal, with 1,500 beds. In addition there are several dispensaries along the line of the Canal. These hospitals were in part located and built by the French. They consist of a series of wooden buildings built on piles and are entirely mosquito-proof (Pl. XII, fig. 6). The buildings are spread out after the fashion of a fever hospital in this country, and the grounds are beautifully laid out. These buildings, like all other American dwellings on the Canal Zone, are for the most part two stories high. A few are three stories in height and many only one storey. They are cool, airy and well shaded. The wire-screening as a rule goes all round the building (fig. 6), but in some cases, especially in the smaller buildings, only two or three sides are screened. The remaining sides consist of wood, with one or two screened windows. The outside walls, so to speak, are therefore composed of wire screening in a wooden framework. The roof consists of corrugated zinc or tile. The eaves, which have no gutters except for the part immediately above the entrance to the house, project about four feet beyond the screened framework, and the water from these falls into a concrete open ditch all round the house. Inside the wire-screening is a space varying from about three to four yards wide, forming a sort of protected

verandah. Then comes the house proper with sitting-rooms and bedrooms, or wards in the case of a hospital building. The walls of the house proper consist of wood with doors and large lattice windows, so that air or wind can pass through the whole building. The rain practically never gets within the wire screen on account of the projecting eaves, and the inhabitants live for the most part in the verandahs immediately within the wire-screening, and therefore practically in the open air. The doors forming the entrance to the buildings are usually double, and consist of a skeleton framework screened with wire. They have strong springs attached so that they shut automatically. The screening consists of pure copper gauze, eighteen meshes to the inch, and lasts as a rule for three years or more.

The hospitals are as well equipped and up to date as any European hospital. All the nurses are white, and each separate building has a laboratory attached with up-to-date microscope and other necessary equipment. The pathological department is likewise large and up to date. At least two of the medical men are employed chiefly on research work.

I feel sure that Ancon Hospital is the most modern and best-equipped hospital in the whole of the tropics. There is only one criticism, it seems to me, to be made regarding the anti-malaria work on the Canal Zone at Panama, namely, that the malarial patients are not subjected to a sufficiently long treatment with quinine. Malarial patients (non-American) remain in hospital only for a few days after their attack of fever has subsided under quinine treatment, and hence a relapse is almost certain. Many patients come to hospital several times a year for malaria, and in the present state of sanitation, it seems improbable that each of these attacks is likely to be a fresh infection. In fact, I am inclined to believe that probably 80 per cent. of the cases of malaria now occurring on the Canal Zone are relapses due to an insufficient course of quinine treatment. The mild character of the cases coming into hospital and the number of re-admissions lead me to this belief. This fault does not lie with the medical men, but is due to the fact that the patients (non-American) do not receive their pay during their stay in hospital, and therefore they are anxious to leave as soon as they feel well, before being properly cured. I believe that a three weeks'

course of thorough quinine treatment safeguards against relapse in most cases. If such a treatment could be given to all patients before they were allowed to leave hospital, I feel sure that malarial fever among the workers on the Canal would finally disappear. The American patients, as a rule, receive a thorough course of treatment before leaving hospital, and malaria is now practically non-existent among them.

RESULTS OF THE SANITATION ON THE CANAL ZONE

In the introduction I have already mentioned the remarkable improvement in health and comfort brought about since the American occupation.

With regard to the remarkable statistical results of the sanitation in Panama, I will again quote from Colonel Gorgas (1911), as follows:—'The health conditions at Panama when the United States took charge in 1904 were very bad. For four hundred years this Isthmus had been considered the most unhealthy spot in the world and the mortality records will sustain this opinion. . . . At one time the construction company of the old Panama Railroad imported 1,000 negroes from the West Coast of Africa, and within six months these had all died off. At another time, for the same reasons, they brought over 1,000 Chinamen and within six months these had all died off. One of the stations at present on the Panama Railroad is called Matachin. The tradition is that this name is derived from the Spanish words mata, 'killed,' and chin, 'Chinamen,' because this was the point where the 1,000 Chinamen were housed and where most of them died. . . . The French lost 22,189 labourers by death from 1881-1889. This would give a rate of something over 240 per thousand per year. . . . Our maximum rate in the early days was 40 per thousand; our rate at present is 7·5 per thousand. . . . The malaria sick rate we have reduced from 821 per thousand to 187 per thousand. But most important of all, yellow fever has been entirely banished. We have not had a single case since May, 1906. . . . The sanitation has cost less than 1 per cent. of the total appropriation for all purposes. . . . We hope that our success at Panama will induce other tropical countries to try the same measures; and that

thereby gradually all the tropics will be redeemed and made a suitable habitation for the white man. But if this is to come about, it must be shown that it can be done at reasonable cost and within the moderate means of the tropical region. . . . I wish therefore to say most emphatically that considering the results and difficulties surrounding the subject, the sanitation of Panama has not been costly. When the Canal shall have been finished it can be shown that sanitation cost about 365,000 dollars (£73,000) per year. For a population of 150,000 this means an expenditure of about 1 cent (one halfpenny) per caput per day, and this sum is well within the means of any tropical country.'

In another article (1910) he made the following interesting statements:—'But I do not believe that posterity will consider the commercial and physical success of the Canal the greatest good it has conferred upon mankind. I hope that as time passes our descendants will see that the greatest good the construction of the Canal has brought was the opportunity it gave for demonstrating that the white man could live and work in the tropics, and maintain his health at as high a point as he can, doing the same work, in the temperate zone. That this has been demonstrated none can justly gainsay. . . We therefore believe sanitary work on the Isthmus will demonstrate to the world that the white man can live and work in any part of the world and that the settling of the tropics by the Caucasian will date from the completion of the Panama Canal.'

SANITATION IN TRINIDAD

On my return from Panama I was able to spend seven days in Trinidad and four days in British Guiana, and although the time was too short to look thoroughly into the question of sanitation in these places, yet in that short period I was able to gain at least some superficial information. My account of the sanitation in these latter places is therefore much shorter than I would have liked.

With regard to malaria in Trinidad, the report of the Surgeon-General for 1911-1912 shows that this disease stands third in the list as a cause of death. The number of deaths from malaria in that year was 722. This is sufficient to show that malaria is very prevalent,

yet very little anti-malarial work is carried on there. One is bitten by mosquitos frequently, yet there are practically no mosquito-proof houses on the island except those of the American Asphalt Company and some of the oil companies. Only one Government residence has been made mosquito-proof, namely, that of the District Medical Officer at Erin. A residence for the District Medical Officer at La Brea is about to be erected, but we believe that the Finance Committee refused to vote the necessary funds for screening it. Furthermore, neither funds nor encouragement are forthcoming to eradicate even the smallest Anopheles breeding places. Some oil and larvicide are employed in some breeding places about the Port of Spain and at a few other places, but this is only done spasmodically.

There are practically no men employed in anti-mosquito work except in Port of Spain, where *Stegomyia* reduction against yellow fever is persistently and more or less successfully pursued, but much more might be done if more trained and efficient Sanitary Inspectors were employed. There are five Assistant Sanitary Inspectors in Port of Spain and three or four for the rest of the colony, but how far these have been trained in tropical sanitation I am unable to state.

With regard to anti-yellow fever work, there are no legal powers to enforce the abolition of eaves gutters, which are an incessant source of *Stegomyia* breeding.

Water barrels are rarely in evidence in the town because of the ample pipe-borne water supply. This water supply, by the way, is not above suspicion, as a considerable epidemic of typhoid fever occurred last year. The streets in Port of Spain are beautifully laid out and well paved, but there is no proper law with regard to garbage disposal, and I understand that proper garbage receptacles, with metal covers, are not provided by the General Board of Health.

Plague is endemic, and large sums of money are spent on rat extermination, etc., yet all kinds of filthy house garbage are placed in open boxes, old baths, barrels or baskets on the pavements, and their contents are liable to be scattered about by fowls, dogs, etc.

There is much ankylostomiasis on the island, yet no estate provides latrine accommodation for its coolie labourers, whether indentured or not.

I need hardly point out, therefore, that in sanitation Trinidad is far behind Panama. This is not the fault of the medical staff, who are constantly urging the advisability of improvement in all these matters. The fault lies with the municipalities and those in power, who are slow to act or to give support either morally or financially.

SANITATION IN BRITISH GUIANA

I have more praise for British Guiana, not because it is more healthy than Trinidad, but because one can see more serious efforts being made there with regard to sanitation than in the latter. British Guiana is an exceptionally difficult place to deal with. All the sea coast, including Georgetown the capital, lies below the level of the sea at high water. A sea wall keeps out the sea during high water, and numerous canals empty their contents through the gates at low water. These canals are essential to drain off the water which collects on this low-lying land. They cannot possibly be dispensed with, but fortunately they are kept comparatively free of mosquito larvae by the swarms of little fish which live in them. Georgetown swarms with mosquitos, one cannot avoid being bitten. It is a large town, beautifully laid out like a garden city, and most of the mosquitos there have been bred in the town itself, or at least very close to it. There are no mosquito-proof houses in the town or elsewhere, and every house has rain gutters on the eaves, leading into barrels or large tanks, as this is practically the sole water supply for drinking purposes. These rain gutters are a constant source of supply of mosquitos. Dr. Wise (1911) has found that 58·3 per cent. of the premises in Georgetown are breeding grounds for mosquitos, the great majority of these being *Stegomyia fasciata*. Several of the streets in the town also have old canals running through them (Pl. XIII, fig. 10). These are a serious defect on account of mosquito breeding. Several of them have been filled in by town rubbish (fig. 11), and finally converted into very fine avenues (fig. 12). Unfortunately, however, the amount of material available for filling in is small, so that these old canals are only being filled slowly, and there are many of them. The land around for miles is so flat that if material were dug out for filling these canals, it would simply mean the

formation of other pools where the material was obtained. With regard to the sugar estates, which employ thousands of coolies, the anti-mosquito problem is even more difficult. The only alternative is prophylactic quinine. In British Guiana, therefore, a decided effort is being made to counteract malaria in spite of the difficulties, but they fall far behind Panama in these efforts.

The Americans cleaned up their towns in Panama thoroughly and at once. It would be wisest in the end to do the same in Georgetown. The old canals in the town ought to be filled in without delay. Material cannot be obtained from the land, but it could easily be obtained from the muddy sandy river mouth by means of quite a small suction dredger. A single steam shovel could also do marvels in the soft land, in making further drainage canals in the surrounding country if necessary. During my short visit the floods were very extensive, and it was quite evident that the canals could not cope with the rainfall.

Concerning the water supply and sewage system, they should keep the example of Colon before them. It seems to me that in the long run it would pay to lay down a pipe-borne water supply, even though it had to be brought a long way. After all, water tanks and rain gutters are a makeshift system and must give way to a better some day. The sewage system is a very difficult problem, yet it was solved in Colon by raising the level of the town.

In Georgetown I am glad to be able to state that the streets are well paved, and that even in heavy rain there are no puddles. Cement open gutters run all over the town. The money for this purpose had to be borrowed, but the improvement brought about is immense. There is not a single mosquito-proof house in the town, but water barrels and tanks are carefully proofed so that mosquitos cannot breed in them. This is excellent, but there are still numerous cans, bottles and other rubbish, suitable as breeding places for mosquitos, lying about in yards, especially about the native dwellings. This should be immediately remedied.

With regard to the sugar estates, all water supply barrels are mosquito-proofed (fig. 13), and ankylostomiasis is counteracted by a good latrine system (fig. 14). In addition, a very large amount of quinine is given as a prophylaxis against malaria to the coolies. The result of this has been to reduce the malaria rate to about one-

third of what it was originally. A good cold storage building has been constructed, steps have been taken to improve the abattoir, and a trained sanitary inspector has recently been obtained for the town. Good work is therefore being done, but the great example of Panama shows that it is possible to do much more, and more should be attempted. For further details of the work being done in British Guiana see papers by Dr. K. S. Wise (1911), Dr. E. P. Minett (1912), and Wise and Minett (1912); also the reports of the Surgeon-General (1912). The majority of the medical men in both of these colonies are thoroughly progressive, but unfortunately their progressive ideas are not always duly appreciated. The sanitary organisation in these colonies cannot be compared with the extraordinarily thorough system in Panama. I have stated that there are a few doubtfully trained sanitary inspectors in Trinidad, and only one recently appointed in British Guiana. The medical men have, in consequence, to do practically everything single-handed. It is extraordinary that, alone, they have been able to do so much. The medical men unaided cannot organise such a system as that in Panama. That is a matter which requires the aid of the community and those in authority. Colonel Gorgas has stated emphatically that his system has paid financially and that, furthermore, the money required is well within the means of any tropical country.

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EXPLANATION OF PLATES

PLATE XI

Fig. 1. Old drainage ditch, Colon.

Fig. 2. Street scene to-day, Colon.

Fig. 3. Early street scene, Colon.

Figs. 4 and 5. Thirteenth Street, Panama, before and after the sanitary improvements. All the streets have been renovated in the same manner.

Photos after Avery & Garrison, Panama.

PLATE XII

Fig. 6. Mosquito-proof building (Nurses' Home), Ancon Hospital, Panama.

Fig. 7. Native labourers of sanitary staff clearing a swamp preparatory to application of oil. Canal Zone, Panama.

Fig. 8. 'Hydraulic Fill,' Canal Zone, Panama. Only tops of trees visible. Sand and mud not yet dry.

Fig. 9. Ancon cemetery, Panama. Graves of French victims.

Plate XIII

Fig. 10. Old canal in Georgetown, British Guiana, not yet filled in.

Fig. 11. Old canal, Georgetown, British Guiana, which has been filled in with town rubbish.

Fig. 12. Old canal, Georgetown, British Guiana, which has been filled in and transformed into an avenue.

Fig. 13. Coolie range, Diamond Sugar Plantation, Demerara, showing water barrels which are all mosquito-proof.

Fig. 14. Latrines for the coolies at Diamond Sugar Plantation, Demerara.

THE CULTIVATION OF ONE GENERATION OF BENIGN TERTIAN MALARIAL PARASITES (*PLASMODIUM VIVAX*) IN VITRO, BY BASS'S METHOD

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PLATE XIV

INTRODUCTION

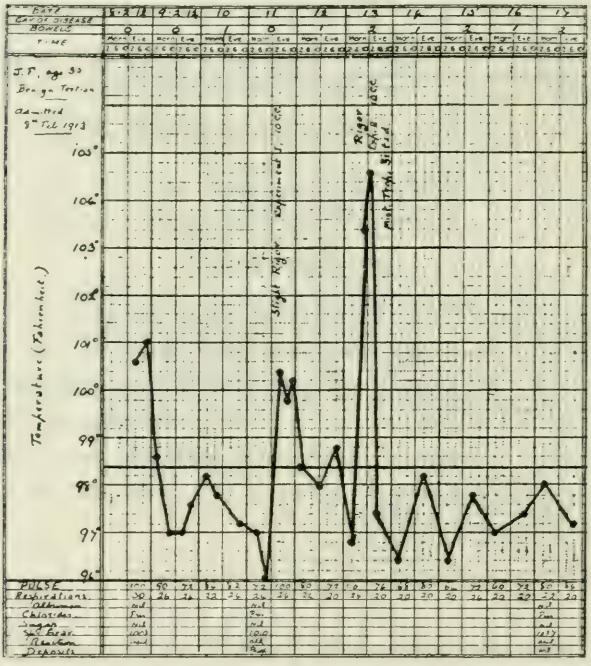
In this Journal (Vol. VI, No. 4, Dec. 1912) J. G. Thomson and S. W. McLellan described the cultivation of one generation of *Plasmodium falciparum*. We are now able to give the following details of the cultivation in vitro of one generation of *Plasmodium vivax*. It may be mentioned that this parasite was cultivated up to three-quarters growth by Dr. H. Carter and one of us (D. T.) in Panama in December last.

CASE

J. F., male, aged 35, was admitted to the Royal Southern Hospital, Liverpool, to Dr. Lloyd Roberts's clinic, in February, 1913.

The patient had resided for some time in the Island of Java, where he developed fever, with vomiting. On admission to hospital a history of four weeks' illness was given, and he complained of rigors, fever, aching pains in the limbs and back, and severe vomiting. Examination of his blood showed numerous young ring

parasites. The temperature on admission was 101° F. It dropped, without quinine treatment, and on the third day another rigor occurred, the temperature rising to 100·4° F. Ten c.c. of blood were taken, while the temperature was at its height, for cultivation of the parasites. Next day the fever had subsided, but on the following day another severe rigor occurred, the temperature rising to 104·6° F. Ten c.c. of blood were again taken from a vein, at the height of the fever, for purposes of cultivation. Quinine was then given in doses of ten grains thrice daily by the mouth, and no further attacks of fever resulted, the patient making a good recovery. The temperature chart is given below.



TECHNIQUE

As stated, 10 c.c. of blood were drawn, with aseptic precautions, from the median basilic vein on two occasions, at the time when the temperature was at its height, on the 11th February and again on the 13th February (see chart).

The blood on both occasions was transferred to a large sterile test tube and defibrinated; it was then distributed into several smaller test tubes and incubated at 39° C. The corpuscles settled gradually, leaving about one half-inch of clear serum above them.

The amount of dextrose solution added was slightly more than the amount recommended by Bass (1912).

EXAMINATION OF CULTURES

Experiment I. A blood smear, taken at the time of the withdrawal of the first 10 c.c. of blood on the 11th February, showed 85 per cent. of young ring parasites and 15 per cent. of three-quarter grown forms, also a few gametocytes. After nineteen hours' incubation there were only 3 per cent. of young rings, 17 per cent. were one-quarter grown, while 70 per cent. were one-half to three-quarters grown, and 10 per cent. showed signs of presegmentation. After twenty-three hours the majority of the parasites were three-quarters grown.

No further examination was made till the forty-fifth hour of incubation, and it was then found that 80 per cent. were small rings, and 20 per cent. were one-half to three-quarters grown. Sporulation had, therefore, occurred between the twenty-third and the forty-fifth hours of incubation.

Experiment II. Blood smears taken at the time of the second withdrawal of 10 c.c. of blood, on 13th February, showed 96 per cent. of young rings, the remaining 4 per cent. of the parasites being about one-half grown (Pl. XIV, figs. 1-3). After eight hours' incubation, 4 per cent. were young rings, 80 per cent. were one-quarter grown, and the remaining 16 per cent. were three-quarters to full-grown (figs. 4-10). After twenty hours, 65 per cent. were one-half to three-quarters grown, with only 2 per cent. of young rings, and a few sporulating forms (figs. 11-17). After twenty-nine hours, 89 per cent. were young rings (fig. 18), 8 per cent. were three-quarters grown, and again a few sporulating forms were found. It is, therefore, evident that in this experiment the maximum sporulation, which we again unfortunately missed, occurred between the twentieth and twenty-ninth hours of incubation.

Although the blood was drawn during the height of the temperature, that is, when the majority of the parasites would have

been expected to be very young, yet they attained their full development and sporulated in little more than half the time which would have been thought requisite for this development.

This, however, is in accord with the results obtained in the cultivation of *P. falciparum* by J. G. Thomson and McLellan (1912). They found that sporulation occurred after twenty-six hours of incubation. We do not think that the 96 per cent. of young rings shown at the time the blood was drawn could have been twenty hours old, so that it must be concluded that growth took place more rapidly in the tube than it would have done in the circulating blood of the patient.

MORPHOLOGY OF THE PARASITES IN CULTURES

The following remarks refer to Experiment II, and are illustrated by the accompanying plate (Pl. XIV). The morphology of the benign tertian parasite (*Plasmodium vivax*), as it occurs in the peripheral circulation, is well known. Unlike the malignant parasite, all stages may be found in the peripheral blood. In malignant tertian, as a rule, only ring forms are found, as the further development of the parasite takes place in the capillaries of the internal organs. In the benign tertian several stages of the development are usually found in a single smear of peripheral blood. Even the full-grown parasites of benign tertian, which, by the way, are much larger than the full-grown malignant *Plasmodia*, are not arrested in the fine capillaries of the internal organs, but are apparently able to circulate freely. A pure case of benign tertian malaria, in which the parasites are all in the same stage of development, is very rarely obtained, yet, as a rule, the majority of the *Plasmodia* are found to be of the same age. In the cultivation of *Plasmodium vivax*, therefore, if a full-grown parasite, or even a sporulating form, is found, it must not be concluded that development has taken place in the culture tube. In order to avoid being deceived by this, we have made careful differential counts in the smears taken at different periods of cultivation.

In our cultures the blood was drawn shortly after sporulation of most of the parasites, so that the majority were young rings. Figs. 1 and 2 show corpuscles with young rings, and already the corpuscles contain many Schüffner's dots, and are somewhat

enlarged, having a diameter of 9μ and 10μ respectively. In fig. 3 the corpuscle is 11·5μ in diameter, and contains a parasite rather more than one-quarter grown.

After eight hours' incubation the blood was again examined, and it was found that the parasites had increased in size in a very marked degree; the majority were now one-quarter grown, and many were three-quarters to full-grown. Fig. 5 shows a parasite about one-quarter grown. Figs. 7 and 8 show half-grown parasites with scattered pigment and the chromatin greatly increased in quantity. Figs. 9 and 10 represent full-grown parasites, and show the collection of pigment into a loose mass and marked increase of chromatin.

In twenty hours the majority of the parasites were half, three-quarters, or full-grown, and several were sporulating (figs. 11-17). Fig. 15 shows the parasite with chromatin divided into seven particles, or daughter portions. Figs. 16 and 17 show typical sporulation, with production of 14 or 15 merozoites. In culture it appears that the number of spores produced in benign tertian is less than that produced in the malignant form of the parasite (Thomson and McLellan, 1912). The maximum number of spores in *Plasmodium vivax* averages about sixteen, but in some cases a few more may be produced.

The culture was not again examined until the twenty-ninth hour, and it was found that the majority of the parasites were then young rings (fig. 18). There was, therefore, evidence that sporulation had occurred and the young merozoites produced had entered new corpuscles. No further asexual development took place.

DISCUSSION OF RESULTS

In the paper by J. G. Thomson and McLellan (1912) it was noted that the parasites, after an incubation of twelve hours, showed a definite increase in size, the pigment being collected together into a circular compact mass. In twenty-four hours the malignant tertian parasite was found to undergo segmentation. The maximum number of merozoites counted was thirty, and it is quite probable that thirty-two is the greatest number of merozoites produced by *Plasmodium falciparum* after complete segmentation. In their remarks, J. G. Thomson and McLellan pointed out the

great difference of opinion among various observers regarding the number of spores produced by the segmentation of the malignant tertian parasites, and reasons were given for this.

In some slides of smears of the spleen and bone marrow made during autopsies of several cases of comatose malaria (*P. falciparum*), sent by Dr. James from Panama, very numerous sporulating parasites were found. The number of spores in these preparations was counted by Sir Ronald Ross and one of us (D. T.). The following table gives the number of spores found in one hundred fully-developed malignant tertian parasites (*P. falciparum*) from a spleen smear of a case of untreated comatose malaria, Panama:—

13	per	cent.	contained	32	spores.
2	,,	,,	,,	31	,,
11	,,	,,	,,	30	,,
7	,,	,,	,,	29	,,
25	,,	,,	,,	28	,,
3	,,	,,	,,	27	,,
17	,,	,,	,,	26	,,
2	,,	,,	,,	25	,,
10	,,	,,	,,	24	,,
3	,,	,,	,,	23	,,
5	,,	,,	,,	22	,,
2	,,	,,	,,	20	,,
100	,,	,,	,, from 20 to 32 spores.		

These numbers lead us to conclude that the maximum number of spores, after complete segmentation, may be as high as thirty-two. This is entirely in agreement with the findings in culture by J. G. Thomson and McLellan, and is a higher number than has, up till recently, been given. Some observers have put the number as low as eight to ten (Stephens and Christophers, 1908).

Again, in a placenta smear from a case of comatose malaria, *P. falciparum*, Panama, sporulating parasites were found which did not fill the entire corpuscle. The average number of spores found in these was only eight. The patient, however, had received 60 grains of quinine during the twenty-four hours preceding death, so that the sporulation was either atypical due to the quinine, or else the parasites had not reached their maximum development. Dr. James, of Panama, also believes that those forms containing few spores are not completely developed, or are atypical sporulating forms due to the action of quinine.

It may now be stated with confidence that the malignant tertian parasite is capable of producing, under favourable conditions, a maximum of thirty-two spores, as proved both by culture and by examination of autopsy smears of cases of untreated comatose malaria. Last year Sir Ronald Ross taught his students that the maximum number of spores produced by the malignant tertian parasite was 2^5, while that of the benign tertian parasite was 2^4.

Another point of great importance, noted in the cultivation of *P. falciparum* by Thomson and McLellan (1912), was that there was a great tendency to clumping of the parasites, both before and after segmentation. This point is of great interest since we have found no such tendency in the case of cultures of benign tertian. This phenomenon explains why the malignant tertian parasites accumulate in the capillaries of the internal organs after attaining a certain size, only the corpuscles containing the young forms being able to circulate in the peripheral blood. It would also explain the blocking of the capillaries in the brain in the comatose form of the disease.

In our cultivation of benign tertian, as already stated, no such tendency to clumping of the parasites has been noted, and they always remain well distributed throughout the smears. This observation is in accordance with what we should expect, since in the benign tertian there is little tendency to blocking of the capillaries, with the resulting coma and other pernicious symptoms. The corpuscles containing all stages in the development of this parasite are able to circulate in the peripheral blood.

Another marked feature to be made out in cultivation is the distribution of the pigment. In the figures given by J. G. Thomson and McLellan for malignant tertian, it is clearly shown that the pigment collects into a circular compact mass before segmentation occurs, and remains so until the complete formation of merozoites. This has also been seen in smears from the internal organs. In the cultivation of *P. vivax* the pigment does not collect so early in the growth of the parasite, and at no time does it appear to form so dense or compact a mass as found in cultures of *P. falciparum*.

There was no evidence of haemolysis in the culture tubes.

POSSIBLE FORMATION OF GAMETOCYTES IN CULTURES

In a paper by one of us (D. Thomson, 1911), it was concluded that crescents develop from the youngest forms of the asexual parasite when a certain amount of immunity or resistance towards the latter had developed in the human blood, and that the time required for their development into an adult gametocyte was about ten days. Reverting now to a consideration of the cultural development of *P. vivax*, it was found that only one generation of asexual parasites developed in the culture tubes. After the first sporulation the parasites remained small, and did not pass through another asexual phase. On the 5th day, however, it was noticed in our cultures (*P. vivax*) that the parasites had changed much in appearance, since all of them had become heavily pigmented and compact (figs. 19-22), *even the very youngest forms contained pigment* (fig. 19). There were no amoeboid straggling forms, all were round and compact, and varied in size from very small circular pigmented bodies to forms as large as 4μ to 5μ in diameter (fig. 22). The pigment was scattered more or less evenly throughout, and the chromatin consisted of a single mass (fig. 21). The protoplasm had a greyish-blue colour, quite unlike that of the asexual phase. On the eighth day there was a very marked increase in the number of large forms, varying from 4μ to 6μ in diameter (figs. 23, 24). All were circular, compact, and very heavily pigmented. On the tenth day the cultures had dried up too much to permit of a further examination.

We are doubtful as to the true explanation of these bodies. They suggest gametocyte formation from their appearance, being circular, compact, and heavily pigmented, and there were many more large forms on the eighth day (figs. 23, 24) than on the fifth day, which would appear to indicate that these bodies had been growing in size. On the other hand, we must consider the possibility that these are degenerating parasites, though it is difficult to understand why degenerate parasites should develop such a large amount of pigment, or why they should become compact and show an apparent slow growth. We were unable to obtain any flagellation in these bodies, and the corpuscles containing them appeared to be shrunken.

SUMMARY

1. The benign tertian malarial parasite is capable of being cultivated up to the stage of sporulation, for at least one generation.

2. In our cultures the growth of the parasite from young rings to sporulating forms took place more rapidly than in the blood of the patient.

3. The cultures of benign tertian differed from those of malignant tertian in that there was no tendency to clumping of the parasites in the former, either before or during sporulation.

4. This difference appears to us to explain in a satisfactory manner why only young forms of malignant tertian are found in the peripheral blood, as the clumping tendency of the larger forms causes them to be arrested in the finer capillaries of the internal organs. It also explains the tendency to pernicious symptoms, such as coma, in malignant tertian malaria. All stages of the benign tertian parasite are found in the peripheral blood, and there are seldom pernicious symptoms, because there is no tendency to clumping.

5. Heavily pigmented, compact parasites were found in our cultures of benign tertian on the fifth day. On the eighth day these had grown larger, and their appearance suggested the development of gametocytes.

6. The malignant tertian parasite (*P. falciparum*) is capable of producing, in maximum segmentation, thirty-two spores. On the other hand, benign tertian (*P. vivax*) produces, as a rule, during maximum segmentation, sixteen spores; sometimes more may be produced, but the number is never thirty-two.

7. The pigment in *P. falciparum* collects into a definite, circular, and very compact mass early in the growth of the parasite. On the other hand, during the growth of *P. vivax* the pigment remains scattered in definite granules throughout the body of the parasite, till just before segmentation, when it collects into a loose mass of granules in the centre of the full-grown *Plasmodium*.

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EXPLANATION OF PLATE XIV

The figures were drawn with an Abbé camera lucida, using ocular 4 and a Leitz 1/12th-inch oil immersion objective, from Romanowsky stained preparations. The pigment is represented by coarser dots in the protoplasm of the organism. The magnification is 1,600 diameters, approximately.

Figs. 1 and 2 represent young plasmodia from the peripheral blood before incubation, showing the corpuscles just beginning to enlarge. Schüffner's dots are well seen; no pigmentation of the parasite is observed.

Fig. 3. Parasite is rather more than one-quarter grown. Corpuscle gradually increasing in size. Chromatin increasing in quantity. Schüffner's dots present. Drawn immediately before incubation.

Figs. 4-10 represent parasites after eight hours' incubation, showing marked increase in size. Forms varying from small rings to the full-grown parasite. Figs. 7 and 8 represent half-grown forms. Figs. 9 and 10 represent practically full-grown forms. The parasites are now distinctly pigmented, and in figs. 9 and 10 the pigment is tending to collect in the centre of the parasite into a loose mass. Note also the great increase in the amount of chromatin.

Figs. 11-17 show parasites drawn after twenty hours' incubation. Figs. 15 to 17 show sporulating forms, the chromatin being divided into fifteen daughter portions in fig. 17.

Fig. 18. Young parasite after twenty-nine hours' incubation.

Figs. 19-22. Highly pigmented small parasites in culture of five days' incubation, suggesting the formation of young gametocytes.

Figs. 23-24 show parasites from eight days' culture. These are highly pigmented, and have increased in size. They are probably young gametocytes.

FULMINATING CEREBRO-SPINAL MENINGITIS IN JAMAICA*

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INTRODUCTION

Three short official reports have been already devoted to this subject, namely: (1) On September 16th, 1912, in connection with certain obscure cases of so-called 'Vomiting Sickness' which suddenly attacked a family in Franklin Town (Kingston) and terminated fatally in four children within a space of twelve hours.

(2) On December 25th, 1912, when reporting upon the result of an examination of cases of 'Vomiting Sickness' at Porus, in Manchester.

(3) On January 7th, 1913; a further report on cases occurring in the same district (Porus), where the disease had apparently taken on an epidemic form.

Before entering on a description of the cases with which we are essentially concerned, I should like to mention four cases (two in April, one in June, and one in July, 1912) which were typical of the ordinary sub-acute variety of Cerebro-spinal Meningitis, and which, so to say, led up to the subsequent investigations. The first was a female child of 12 months, and the duration of illness was three weeks; the second was a male, aged 3 years, who lived for 6 days; the third was a lad of 17 years of age, ailing for a fortnight or so before the onset of any typical symptoms, and death took place a fortnight later; the fourth, a woman of 19 years, who was ill for fully three weeks, possibly longer. This also terminated fatally.

* This paper contains observations made up to Jan. 9th, 1913, at which date Dr. Harald Seidelin, on expedition for the Liverpool School of Tropical Medicine, arrived in Jamaica in order to investigate the so-called Vomiting Sickness. Dr. Seidelin suggests that these observations should be published in order to make known the work which had been done previously to his arrival.—H.H.S.

In all four a pure growth of meningococcus was obtained on nasgar, and proved by sub-culturing on the same medium and on ordinary agar, and incubating at 37° C. and at 25° C. Growth occurred only at the higher temperature, and in no case on ordinary agar until the third or fourth sub-culture. Maltose, dextrose and galactose were fermented, saccharose, lactose and mannite were unchanged.

SOME CASES IN KINGSTON

No similar cases came to my notice between July and September. On the 4th of the latter month a sudden outbreak of cases of a very acute nature occurred in Kingston itself, of which the following is a brief history, as reported on the 16th.

A cigar-maker, named Adolphus Peart, his wife and seven children, lived in a small house of two apartments, No. 6, Norfolk Lane, Franklin Town. Three of the children, named Constantine, Adolphus and Ruby, aged 8, 5 and $3\frac{1}{2}$ respectively, were a little 'out of sorts' and had slight attacks of vomiting during Wednesday, September 4th, but were not, to all appearances, sufficiently ill to call for any treatment, and at night the whole family retired in ordinary health. About midnight, the youngest (Ruby) began to vomit, and shortly afterwards Adolphus and Constantine did the same. This, however, seems to have ceased temporarily and the children fell asleep again. In the early morning the second child (Adolphus) woke up, asked for some food, but, before having any, was seized by a convulsive attack; the other two (Constantine and Ruby) within a short period were similarly convulsed, and all three lapsed into a state of coma. A doctor was called in, but shortly after 7 a.m. the one first attacked (Adolphus) died. The other two were brought to the hospital at 9 a.m., suffered at intervals of a few seconds from convulsive seizures, the spastic condition of the neck in the case of the older patient, at all events, not completely relaxing even in the intervals, and the younger (Ruby) died at 10 a.m., the older (Constantine) at 10.55 a.m., without recovery of consciousness in either case.

In the meantime, the eldest girl of the family, Ethel, aged $14\frac{1}{2}$ years, started to vomit and within a few minutes became, to a

certain degree at least, unconscious. She was also brought to the hospital, arriving at 9.30 a.m. When I saw her at 10.30, she had recovered consciousness, but showed rigidity of the neck (and possibly of the spinal muscles) and, while being examined, had a convulsive attack; these were repeated, but were not so severe as in the case of the boy. Nevertheless, the patient sank more deeply into coma, and died at 5.55 p.m. Thus, four of the children had died between 7 a.m. and 6 p.m.

No dietetic error could be discovered in any of the cases, and, apparently, the only previous history obtainable was of a 'cold in the head' for two or three days preceding. This, however, had been disregarded as the condition is common in the city.

Post-mortem examinations were held in all four cases; the details of three of them performed by me will be recorded shortly, after the history has been completed.

At the funeral of these four on September 7th, another sister, Violet, aged 11 years, suddenly complained of headache, vomited, and became collapsed and semi-conscious. She, too, was hurried off to the hospital. She had recovered consciousness by the time she arrived, but showed a slight degree of rigidity of the neck muscles; Kernig's sign present on the right side, but less pronounced or doubtful on the left. The vomiting was effortless, and not accompanied by marked nausea; the pupils were equal and somewhat dilated. This patient made a good recovery.

PATHOLOGICAL DETAILS

Cerebro-spinal fluid was taken by lumbar puncture from Constantine, Ethel and Violet. (Ruby was dead in the hospital, and Adolphus had died at home.) The fluid in the first two cases was clear, in the last-named distinctly turbid. Enumeration of the leucocytes in this last revealed 92 per cent. polymorphonuclar cells, and in several were small Gram-negative diplococci. Cultures were made from all three, and in every case the meningococcus was isolated. The nature of the organism was proved by sub-cultivation on various media and by fermentation tests. The same organism was recovered from the intraventricular fluid of the child Ruby, obtained post-mortem.

The macroscopic post-mortem signs were similar in every case, but more marked in Ethel, who had been longest ill. There was a distinct turbid haziness over the surface and base of the brain, extending down the cord, with excess of fluid (cultivation of this also yielded the organism), while in the case of Ethel the whole surface of the convexity of the brain, and particularly the interpeduncular space at the base, showed a much thicker layer of pearly lymph, and here and there definite flakes of it.

These meningeal signs were common to all, varying only in degree. The only other morbid conditions found were, in the case of Constantine, some oedema of the lungs, and some scattered petechiae on the surface (he had exhibited the most violent convulsions), and in the case of Ethel there were firm adhesions of the left pleura of old standing, and a similar but less marked condition of the right. There were no signs of tuberculosis anywhere in any of the cases.

The viscera of all four were sent in sealed jars to the Island Chemist for examination, but he reported that no signs of any poison were discovered.

The initial 'catarrhal' symptoms were in all probability what has been noted as the 'Premeningeal Catarrh,' set up by the presence of the organism at its 'site of election' in the upper part of the naso-pharynx, whence it spreads by way of the nerve canals through the cribriform plate of the ethmoid to reach the meninges.

The mother, baby, and the son Reuben, were admitted to hospital for observation, and swabs were taken from the upper reaches of the naso-pharynx in each case. The meningococcus was isolated from the boy's throat, not from the other two. He was, therefore, a 'carrier' of the organism, and was detained at the hospital.

The history of these patients is typical of nearly all the acute cases of 'Vomiting Sickness'; in fact, the medical man who sent the patients to hospital stated that had they been attacked during the winter months, he would have described them as typical 'vomiting sickness' cases.

This position is taken up by many physicians practising in country districts, namely, that if they meet with cases in the colder months they call them Vomiting Sickness, but at other times of the

year the deaths are certified as due to worms (which are present in most of the native children), gastro-enteritis, food poisoning, etc.

In my opinion, this idea is responsible for many sporadic cases being overlooked, and it is possible that such carry on the infection through the summer and form the starting point of the localised epidemics in the colder season.

From the cultures of the Peart cases I prepared a vaccine, not so much with a view to curative treatment of subsequent cases, for as a rule the course is so acute that no favourable result from such treatment could be expected, death often occurring in two or three hours after the onset of the first observed symptom, but as a prophylactic in districts where the outbreaks mostly take place.

Summary

1. In four cases presenting the typical symptoms of 'Vomiting Sickness' the Weichselbaum's diplococcus has been isolated.

2. These symptoms correspond to those associated with foudroyante types of Cerebro-spinal Meningitis.

3. The organism was obtained from the cerebro-spinal fluid of all those from whom this fluid was taken during life, and from the cerebral ventricular fluid post mortem.

4. The organism was obtained from the naso-pharynx of one of the contacts of these cases, though himself apparently in perfect health.

5. The intracranial post-mortem signs were such as are consistent with death from Cerebro-spinal Meningitis at such an early stage, that is, with fulminating cases of the disease.

This focus did not spread, and the child (Reuben), who was proved to be harbouring the organism in his naso-pharynx, did not develop the disease.

METHODS OF INVESTIGATION

Having been struck by the peculiar hyperacuteness of these cases, and by the remarkable similarity of the symptoms to those reported from various districts of the island as occurring in the devastating epidemics which arise every cold weather, preparations were made

for thoroughly testing the hypothesis that some at least of these cases might be specially acute Cerebro-spinal Meningitis.

(*a*) Forms of report for filling in the histories of those patients who were seen during life, and special forms for the reporting of post-mortem findings were drawn up and distributed to the various District Medical Officers.

(*b*) Considering that the meningococcus does not grow well on ordinary nutrient agar, sloped tubes of nutrose ascitic agar were prepared, placed in boxes containing also sterilised swabs, slides, and ampoules of vaccine. These boxes were likewise distributed to the District Medical Officers with directions as to the use of the material contained. The tubes of sloped nasgar were for inoculation with cerebro-spinal fluid obtained by lumbar puncture, and for inoculation with material obtained by means of the sterile swab from the upper reaches of the naso-pharynx of contacts. The slides were for smears of the spinal fluid and of blood (as some of the cases might be malarial). The vaccine was for use with contacts for prophylactic purposes.

(*c*) Anti-meningococcal serum was ordered.

Since the organism, presuming that a variety of the meningococcus might be the cause, is far from being a resistant one, it was considered that if the spinal fluid itself were sent, the organism might die before the specimen reached the laboratory, so that cultural tests could not be carried out. In some instances there is an interval of over twenty-four hours between the time of despatch of a specimen and its arrival at the laboratory. Secondly, that if the organisms were present in small numbers only, they might be overlooked in a smear. Thirdly, that if the inoculation of the selective medium were made on the spot, colonies would in many cases develop by the time the specimen reached the laboratory, and examination of these and sub-cultivation could be made the same day, and in this way a considerable saving of time would be effected.

On the whole, I may say that this system has worked very well, since in several instances the tubes on arrival at the laboratory

showed pure cultures, and in others there was sufficient growth to enable plating to be carried out at once for purposes of isolation.

The procedure subsequent to the box and its contents reaching the laboratory has been as follows:—

1. Smears were examined for enumeration of leucocytes and the relative proportions in which they were present, and for observations of any diplococci, intracellular or otherwise. A second smear was stained by Gram's method and counterstained. Sometimes, where nothing definite could be made out from the smears sent of the cerebro-spinal fluid, another smear of the liquid in the culture tube would give indications of the varieties of organisms present.
2. Any likely-looking colonies on the culture media were sub-cultured and examined.
3. The growth in the culture tube was plated for purposes of isolation.
4. The colonies so isolated were then examined, and sub-cultures made on nasgar and ordinary agar and incubated at 37° C. and at 25° C.
5. From the growth so obtained (only the former showed any, in many instances) the effect on various sugar media was noted. Dextrose, maltose, galactose, saccharose, mannite and lactose were generally employed.

GENERAL SYMPTOMS

Returning to the description of the various cases with the examination of which I have been concerned (prior to the 9th of January), it would be tedious, and would unduly prolong this short paper, to give details of the history of each one. The usual train of symptoms is as follows:—

The patient, usually a child, goes to bed apparently in its ordinary health, or there may be a history of slight indisposition, a cold in the head, or some loss of appetite, or a tendency to be 'droopy' and to lie down during the day preceding the actual onset. During the night the child wakes up and vomits—perhaps only once, perhaps three or four times—and complains of feeling ill. After

an hour or so he falls asleep again, and some three or four hours later, more or less, again wakes up, complains of pain in the abdomen (pain is used as a term for mere discomfort frequently among the natives), and almost at once begins again to vomit, usually frothy mucus, occasionally bile-stained, and later only watery fluid, with, in most instances, little or no effort, unless the stomach is quite empty, when troublesome retching ensues; if, however, food or liquid is taken, there is apparently effortless vomiting.

In a very short time, often a matter of a few minutes, convulsions come on, and there is 'stiffness of limbs and drawing back of the head' (as the parents describe it), coma rapidly succeeds and terminates in death.

In some there is no stiffness or retraction, but a general limp condition.

The total duration is short, the average being some four to twelve hours; the most rapid in my experience was thirty-five minutes. Frequently, therefore, the patient is not seen during life, and the history is both incomplete and unreliable, as it is obtained by questioning the parents, who, not having noticed any details of symptoms, will deny their presence, or affirm with equal readiness in order to ingratiate themselves, for the acuteness of the course formerly in many instances gave rise to the suspicion of poisoning.

When seen during life, the child is usually in the convulsive or comatose stage. The temperature is rarely high, usually 101°-102° F., but it may be normal. The pulse-rate is between 90 and 100, fairly strong; respiration 26-30, regular till towards the end, when the Cheyne-Stokes' type may appear. Kernig's sign is present in some of the cases, and may be distinctly more marked in one leg than in the other; rigidity of the neck muscles is more common than retraction of the head, and this rigidity is often overlooked because the flexion is not attempted in the strictly middle line. Rigidity may be fairly marked, but when the flexion is combined with lateral movement (as is the case where the test is applied with the child lying on its side) the stiffness may be masked, since lateral movement may be comparatively free in spite of distinct rigidity of the neck muscles.

The pupils are usually equal, moderately dilated, and, if the

coma is not deep, react normally. In a few there is photophobia, and in those retaining consciousness, general irritability and complaint of headache—not always by any means severe—usually frontal, sometimes general. Delirium is, so far as I have seen, quite uncommon; shortly before passing into the comatose state, the child may remark that it ' feels very bad,' but does not call attention to any particular symptom, or locate the pain, if such is complained of, to any particular spot.

In cases which do not end fatally, the state of coma is rarely present; there is vomiting, headache, convulsions with temporary loss of consciousness only, and recovery is almost as rapid as the onset. Within twenty-four hours a child, who has been seriously ill, may be sitting up in bed, and in twenty-four to forty-eight hours later be up and about, showing practically no symptoms except a little pallor, general debility which rapidly clears up, and some residual headache of no great severity, while others in the family, who did not seem to be any worse at the time, have passed into a state of coma and died in a few hours.

FURTHER CASES

In the following descriptions, where the words ' usual history ' are employed, the course of events has been that sketched above.

The next case, after the Franklin Town outbreak, which came under my own personal notice (the tenth of the series of which I have notes) occurred also in Kingston, on October 1st, 1912. This, too, ran a more prolonged course than the generality of cases here.

(10) Male child, aged 6 years, was apparently quite well on September 25th. During the night he awoke and complained of intense headache, calling out ' My head, my head.' Went to sleep again, but early in the morning of the 26th awoke again and called out with headache, was seized with convulsions and lost consciousness. Slight vomiting only, but much more severe next day.

When admitted to hospital (October 1st) there was definite retraction of the neck, Kernig's sign marked, pupils dilated and equal. Child quite conscious. Cerebro-spinal fluid turbid and

showing well-marked intracellular cocci, Gram-negative and diploid in arrangement, with 84 per cent. of the leucocytes polymorphonuclear.

A good growth of meningococci was obtained, from which a vaccine was made.

During the succeeding three weeks the patient's condition got gradually worse; there was general flexion and irritability, retraction of neck, wasting, etc., but very few twitchings and still some vomiting. There was no serum at hand, so an injection of 100 cocci was administered on October 24th. Note made on the 26th:—'Patient brighter and improving, temperature lower.' On November 7th:—'Improvement steadily maintained; retraction gone, Kernig's sign absent, is getting up and taking plenty of nourishment.' Two days later he went home, and reports state that he is in good health but exhibits violent outbursts of temper on trivial grounds, quite contrary to his former disposition.

This was in onset like the ordinary hyperacute Cerebro-spinal Meningitis, but later took on the characters of the subacute and recovered.

(11) P. McM., female, aged 4 years. History typical, namely, apparently well at 11 p.m., October 12th; early on the 13th was dull and restless (usually very bright and cheery), doubtful headache—child held its head, but did not make any definite complaint of it. About 7 a.m. child had a convulsion with the head thrown back, and general twitching and stiffness. Rapidly lapsed into coma and died without recovering consciousness.

At the autopsy the vessels of the surface of the brain were congested; there was a pearly haze over the hemispheres, with flakes of lymph in two or three spots. Ventricles contained excess of fluid; smear of cerebro-spinal fluid showed intracellular Gram-negative diplococci. Culture obtained on nasgar, and proved by subsequent tests to be the meningococcus.

(12), (13), (14). Three children, sisters, aged 8 years, 2 years, and 9 months, were brought to the hospital on October 23rd, 1912. All three had a history of headache and vomiting, and Amy (the one of 2 years) showed some staggering—she was old enough to walk straight when well—stiffness of neck muscles was present in all, Kernig's sign in the two older, but doubtful in the baby.

Swabs taken from high up the naso-pharynx (lumbar puncture was not permitted) gave meningococcus on cultivation, mixed with *Micrococcus catarrhalis* and some Gram-positive bacilli (Hoffmann), but separated by plating. They were all three seriously ill for the next week or so, especially the two younger, but after that made steady progress towards recovery.

It may be added that the mother of these children complained of headache and of pain along the neck and shoulder, but the organism was not obtained in her case.

(15) J. L. B., male, aged 4 years, October 19th. History given was that which has been described at length above. The duration of illness was only two hours. At the post-mortem examination the 'meninges were markedly congested, fluid in excess in the cerebral ventricles, and at the base of the brain. The fluid was turbid uniformly.' (Quoted from notes sent by Dr. Purchas.) No specimens forwarded, so the cause was not proved.

(16) C. M., male, aged 13 years. Reported December 1st, 1912, from Montego Bay. The only indication of anything which might be regarded as a premonitory symptom was a 'fresh cold' for a week preceding the sudden onset of vomiting at 4 p.m. The patient became unconscious at 6 p.m., convulsions started at 7 p.m. and recurred at intervals till death at 3 a.m. the following day. The total duration was, therefore, 11 hours.

In this case there were a few ascarides found; the mesenteric glands were enlarged. The organs, except the brain, were reported as normal. But here the 'meninges were markedly congested, especially over posterior halves of the hemispheres, and cerebellum, and at the base. Fluid was in excess, turbid and milky.'

The meningococus was isolated from the culture sent.

(17) C. M. A., female, aged 4 years. December 7th. Usual history, except that the patient was said to have retained consciousness till death. The medical attendant states that he did not see the patient during life, and that the 'father's statements as regards the symptoms are unreliable.' Duration of illness 15 hours. Culture of spinal fluid yielded the meningococcus.

(18) H. W., female, aged 6 years. December 7th. Usual history.—First symptom—vomiting—'started early in the morning, and death occurred at 10 a.m.' Smears of spinal fluid gave a

differential leucocyte count of 82 per cent. polymorphonuclear, 13 per cent. lymphocytes and 5 per cent. endothelial cells, and showed Gram-negative diplococci mixed with coliform organisms. Smears of intraventricular fluid gave 85 per cent. polymorphs, 12 per cent. lymphocytes and 3 per cent. endothelial cells, and the same organisms.

The culture was badly contaminated, probably owing to the long interval (fifty-six hours) between death and the autopsy, and I was unable to isolate the diplococci. This case, therefore, was associated with a Gram-negative diplococcus in the spinal fluid, the nature of which was not definitely proved.

(19) A. J., male, aged 2 years. December 19th. Usual history of vomiting, succeeded by coma, but there was no mention of convulsions. Duration of illness six hours. Meninges dull, vessels congested; fluid in ventricles and spinal canal in excess and turbid.

The fluid sent showed Gram-negative diplococci, but they did not grow on the culture medium.

(20) R.T., female, aged 6 years. Porus District. December 22nd, 1912. This is a very good instance of the condition under description, because, having been sent down to investigate a small outbreak at Porus, I was able to see the patient myself during life and to take specimens of the blood and spinal fluid, and to perform the autopsy almost immediately after death took place.

The patient was a well-nourished child, and was said to have been quite well until 6 p.m. on December 21st, when an attack of vomiting came on. There was little or no complaint of headache. After sleeping for several hours, she commenced to have attacks of convulsions at intervals, from 4 a.m. on December 22nd, during which she 'went quite stiff.' When I saw the patient at 12 noon she was comatose. There was a general limp condition of the muscles, but on flexing the head in the middle line the neck muscles felt hard and stiff. Kernig's sign was marked in both legs, but more so in the left than in the right. There was a small group of herpetiform spots on the lips at the right side of the mouth, but no discernible rash on the body.

I took a blood smear and also spinal fluid smears and some of the fluid for culture. There was no recovery of consciousness, and

death took place at 12.30 p.m.; the total duration from the first onset of symptoms being 18½ hours.

All the organs appeared normal except that the mesenteric glands were enlarged, and the brain and meninges were markedly congested; there was a general haziness over convexity and base, more over the right hemisphere, and in parts there was distinct opalesence to milkiness. The ventricles were filled with clear fluid.

The smears of the spinal fluid taken showed many Gram-negative diplococci, several intracellular, but more lying free. The differential count of the leucocytes gave polymorphonuclears 26 per cent., lymphocytes 67 per cent., endothelial 7 per cent. The blood smear showed well-marked diplococci; this is the only case in which I have seen them in a blood smear taken during life, though it is only right to say that I have had very few opportunities of examining blood smears taken shortly before death.

The culture tubes showed several small colonies of Gram-negative diplococci which subsequent tests proved to be meningococci; and in this instance, therefore, not only was there definite Cerebro-spinal Meningitis, but a condition of meningococcaemia was shown to be present.

(21) D. D., male, aged 11 years. December 20th, 1912. The history in this case was typical, except that the symptoms were a little more drawn out. He complained of frontal headache and chilliness on December 18th at 11 a.m., and vomited in all sixteen times during that and the succeeding day. Was walking about in the evening of the 19th, and complained about 11 p.m. that the lamplight hurt his eyes. He was restless all that night, and had frequent convulsive movements of the limbs with retraction of the head and neck, and general signs of cerebral irritation till 4 a.m. on the 20th, when coma supervened, and he died at 11 a.m. The duration, therefore, was forty-eight hours.

Post-mortem report stated that there were some round worms in the intestines and some congestion of the mucous membrane of the stomach, but beyond this the only abnormality noticed was marked congestion of the meninges, and a general dulling and loss of glistening appearance of them, with excess of fluid in the ventricles.

No cultures or specimens were sent from this case, so the proof

of the cause was not forthcoming. The case is described for two reasons: firstly, because several similar cases followed it in which the organism was detected; and secondly, because it is a good example of the partial recovery or comparatively calm interval between a violent onset and an equally violent return of more serious symptoms which usually terminate fatally.

(22) R. E., male, aged 7 years. December 24th. Same district. Usual history. 'Duration 24-30 hours.' Spinal fluid gave typical cultures, and the meningococcus was proved.

The last-mentioned cases were the forerunners of a small epidemic in this district. Details of the individual cases need not be given, as they would be largely a repetition of what has been already stated; nevertheless, there are some points of interest about them, which may be briefly summed up as follows:—

Nineteen cases occurred during the last week of the year 1912, of which notes were sent to me. In nine of these no lumbar puncture was performed, and therefore, however typical the symptoms, they cannot be said to be proved cases of Meningitis. In seven of the nine, however, nearly all the classic symptoms—headache, vomiting, irritability, rigidity, Kernig's sign—were present, so that there is a fairly strong probability that these were instances of the disease; two of them recovered within three days and the rest within a week.

In six of the remaining twelve there was no symptom recorded except vomiting; these rapidly cleared up with a stomachic mixture, and I think they were probably merely gastric disturbances of a mild type.

Lastly, in six of the ten specimens of fluid sent up, the meningococcus was isolated from the spinal fluid and proved; three of them terminated fatally—one aged 7 years was ill for thirty hours; a second, aged $3\frac{1}{2}$ years, died after ten hours' illness; the duration in the third, an adult, could not be ascertained.

Twenty-two subjects, nine of them contacts, and thirteen showing what was thought to be early symptoms of the condition—vomiting (with no ascertainable cause), headache and malaise—were given an injection of the vaccine which had been prepared by me from a former case. Of these thirteen, three were proved by culture from the spinal fluid to be definite cases of Cerebro-spinal Meningitis; of the remaining ten there is no evidence to show whether they were

cases of meningitis, worms, gastro-intestinal upset, or for the matter of that any other disease associated with vomiting, but it may be mentioned that none of the vaccinated contacts have so far been attacked, and none of the suspected or proved cases who had been vaccinated have died.

In the smear of the spinal fluid from a boy of 7 years of age, who died in ten hours, and in whose case the meningococcus was isolated, I found also several spirochaete-like bodies, or long somewhat tapering bacilli. Whether these have any causal connection with the disease I cannot say, but I may mention, in passing, that in two cases in which I have seen these bodies, the course has been exceptionally rapid. The second died in seven hours after the onset of the vomiting. In neither case did this peculiar organism develop on the medium used.

Of eleven cases subsequently reported with symptoms similar to those already detailed, spinal fluid cultures were sent up from seven. In three instances the meningococcus was isolated; in three no growth occurred; in the remaining tube there was a slight growth which had developed during the transit of the culture to the laboratory, but on arrival there after the laboratory had been closed for the day, the box containing it was, in excess of zeal on the part of the recipient, placed on ice, and the organism had been killed, for sub-cultivation failed altogether.

NOTE ON VACCINE TREATMENT

I did not expect any beneficial effects from vaccine treatment in the acute cases of the disease, as has been already stated. The vaccine was intended for prophylactic use mainly, and secondarily for chronic cases should such be met with. For the acute and hyperacute cases the anti-meningococcic serum was ordered, but there are points militating against its employment even in many of these. Firstly, there is the difficulty of keeping the serum in warm climates in districts where no ice is obtainable; secondly, though it has been stored in various parts of the island where ice can be procured, nevertheless, many of the cases are so rapidly fatal that death occurs before the serum arrives, although it may have been telegraphed for on appearance of the earliest symptoms and have been despatched by special messenger without delay; thirdly, the

serum must be injected intraspinally and repeated on three successive days at least, and if the case terminates fatally, the parents are almost certain to ascribe the death to the medical man's action in injecting fluid into the spinal canal.

Apart from the cases in which the vaccine was used prophylactically, I do not think that the conclusion is justified that the happy results following its employment in some of the cases referred to above can be attributed to its use. The condition was too severe for the small dose employed to have much effect, if any; also the amelioration was too rapid and has been too well maintained to justify any inference that the improvement leading to recovery was the direct result of the small injections made. I feel that, though 'post hoc,' there are no grounds for regarding the recovery as 'propter hoc,' at all events until further reports of the efficacy of the vaccine in similar cases have been received.

GENERAL SUMMARY

1. In several cases of illness with sudden onset in apparently healthy subjects, terminating fatally in a high percentage of those attacked, I have isolated a Gram-negative diplococcus from the spinal fluid.

2. This organism gives the morphological and cultural characters of Weichselbaum's *Diplococcus intracellularis meningitidis*, except that in galactose it does not always give a definite reaction, the medium in some instances not being affected. The maltose and dextrose, however, are always typically acted upon.

3. These cases exhibit in many instances a symptom-complex which has for years been spoken of in Jamaica as 'Vomiting Sickness.'

4. The disease breaks out in localised epidemic form in various parts of the island every cold weather, that is, from about the middle of December to the end of February or beginning of March.

5. The disease occurs at other times as sporadic cases, but apparently does not spread extensively.

6. It is a curious thing that practically all the cases are of the hyperacute variety, either recovering in a few days, or dying in a few hours. One rarely sees the subacute cases during the epidemic times, and never, so far as I am aware, the chronic ones, unless

some of the natives one meets with who are chronically deaf, or have seriously defective vision, or who suffer from fits, are instances of Cerebro-spinal Meningitis with permanent sequelae. Of this there is no proof.

7. In two instances a spirochaete-like body, or long curving bacillus has been seen in the smears made directly from the fluid obtained by lumbar puncture. What part, if any, this takes in producing the symptoms I have had no means of discovering.

8. The disease is very rare amongst the white population; I have known of only three instances so far during the past year. Possibly this is due in part to better hygienic conditions, less crowding, and so forth, but not entirely, because

9. I have not personally met with the condition once amongst the East Indian population, where overcrowding and bad hygiene are nearly, if not quite, as marked as in the case of the native. For this, also, I am unable to offer any explanation at present, though it may be noted that the food supply of the East Indian is usually better than that of the West.

Pathological Laboratory,
The Public Hospital,
Kingston, Jamaica, Feb. 18, 1913.

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The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1913:—

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EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the second number of the seventh volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

FINAL REPORT OF THE LUANGWA SLEEPING SICKNESS COMMISSION OF THE BRITISH SOUTH AFRICA COMPANY

1911-1912

BY

ALLAN KINGHORN, WARRINGTON YORKE

AND

LLEWELLYN LLOYD,

ENTOMOLOGIST TO THE COMMISSION

(*Received for publication 7 February*, 1913)

PLATES XV-XXVI

CONTENTS

PAGE

INTRODUCTION ... 184

SECTION I. THE HUMAN TRYPANOSOME—

(*a*) Distribution of the Disease ... 185
(*b*) Clinical Features ... 186
(*c*) Identity of Parasite with *T. rhodesiense* ... 187
(*d*) Transmission of the Trypanosome ... 187
(*e*) Influence of Meteorological Conditions on Development of the Trypanosome in *Glossina morsitans* ... 204
(*f*) Reservoir of the Trypanosome ... 214
(*g*) Occurrence of the Trypanosome in *Glossina morsitans* in Nature ... 215
(*h*) Identity of 'Game' and 'Fly' Strains with the 'Human' Strain of *T. rhodesiense* ... 217
(*i*) Summary ... 224

SECTION II. TRYPANOSOMES OF GAME AND DOMESTIC STOCK—

(*a*) Methods ... 227
(*b*) Examination of Game at Nawalia (Luangwa Valley) and Ngoa (Congo-Zambesi Watershed) ... 228
(*c*) Examination of Domestic Stock ... 235
(*d*) Summary ... 237

SECTION III. TRYPANOSOMES FOUND IN WILD *Glossina morsitans*—

(*a*) At Nawalia ... 239
(*b*) At Ngoa ... 241
(*c*) Summary ... 243

SECTION IV. DESCRIPTION OF TRYPANOSOMES—

(*a*) *Trypanosoma rhodesiense* ... 245
(*b*) *Trypanosoma vivax* ... 245
(*c*) *Trypanosoma nanum* ... 248
(*d*) *Trypanosoma pecorum* ... 251
(*e*) *Trypanosoma multiforme*, sp. nov. ... 254
(*f*) *Trypanosoma montgomeryi* ... 261
(*g*) *Trypanosoma ignotum*, sp. nov. ... 263
(*h*) *Trypanosoma tragelaphi*, sp. nov. ... 269

SECTION V. DEVELOPMENT OF *Trypanosoma rhodesiense* IN *Glossina morsitans*— 273
Summary ... 282

SECTION VI. REPORT OF ENTOMOLOGIST—

(*a*) *Glossina morsitans* in the Laboratory ... 285
(*b*) Breeding Places of *Glossina morsitans* ... 289
(*c*) Bloodsucking Insects collected at Nawalia and Ngoa ... 293

APPENDIX

A. An Experiment to ascertain whether Tabanids transmit Trypanosomes in Nature ... 299
B. An Attempt to Transmit *Trypanosoma rhodesiense* by means of *Ornithodoros moubata* ... 301

INTRODUCTION

During the years 1909 and 1910, the diagnosis of several cases of human trypanosomiasis amongst Europeans, who had never been in contact with *Glossina palpalis*, drew attention to the occurrence of the infection in portions of Rhodesia and Nyasaland in which this particular insect was not known to exist. As the result of prolonged and careful search, it was definitely proved that *Glossina palpalis* did not occur in these areas, and accordingly, in the beginning of 1911, this Commission was instituted by the Chartered Company to ascertain the transmitting agent.

Nawalia, in the Luangwa Valley, was chosen as the site of the laboratory (Pl. XV, figs. 1-2). Work was commenced at the end of June, 1911, and was continued until April, 1912. Our investigations quickly placed it beyond doubt that *Glossina morsitans* was the vector of the human trypanosome, and further revealed the fact that a considerable percentage of game and of 'wild' *Glossina morsitans* were infected with the same parasite.

In April, 1912, the headquarters of the Commission were removed to Ngoa, on the Congo-Zambesi watershed, in order that experiments might be undertaken to ascertain what influence, if any, was exerted by climatic conditions on the transmission of the trypanosome. Work at Ngoa (Pl. XVI, figs. 1-2) was continued until the end of August, 1912, when the Commission left for England. By this time it had been definitely determined that the relatively low temperatures experienced during the cold season on the plateau were inhibitory to the completion of the developmental cycle of the human trypanosome in *Glossina morsitans*.

Records of the greater portion of the work embodied in this report have been published from time to time in the form of separate papers. As, however, these preliminary communications were necessarily somewhat incomplete, it was considered desirable to collect and correlate all the results in the final report.

We desire to acknowledge our indebtedness to Dr. Aylmer May, Principal Medical Officer, Northern Rhodesia; to Dr. A. F. Wallace, M.O., N. Rhodesia; and to E. A. A. Jones, Esq., Assistant Magistrate, Mpika, for the great assistance they rendered the Commission.

The report was completed at the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine.

SECTION I

THE HUMAN TRYPANOSOME

BY

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(*a*) OCCURRENCE OF THE DISEASE

The distribution of human trypanosomiasis, due to infection with *T. rhodesiense*, is comparatively wide. When the parasite was first described,* the Luangwa Valley was the only region known to be implicated, but it has since become apparent that the disease is much more widely disseminated.

In the territory formerly known as North-Eastern Rhodesia, cases have been diagnosed not only in the Luangwa Valley, but also in the districts south of Fort Jameson and to the west of Serenje. It may be mentioned further that this parasite was isolated from wild game in the vicinity of Ngoa, and from a native dog living in a village on the Nyasaland boundary. It is possible, therefore, that sporadic cases of the disease may exist in these localities also, but on this point no definite information can be given.

In North-Western Rhodesia, it would appear that *T. rhodesiense* exists in at least one locality, namely, between Broken Hill and the Anglo-Belgian boundary. The details of a case, due to this organism, and contracted in the district named, have recently been described by Ellacombe.†

It is, of course, occasionally very difficult to ascertain the exact locality in which the infection was acquired, more particularly in native cases, but there are good grounds for believing that those from the districts mentioned were autochthonous.

* Stephens and Fantham, (1910). Roy. Soc. Proc., B, Vol. 83, p. 28. Annals of Trop. Med. and Parasit., IV, p. 343.

† Ellacombe, G. W. Sleeping Sickness Bulletin, 1912, Vol. IV, p. 185.

Beyond the confines of Northern Rhodesia, an instance of infection by *T. rhodesiense* in Nyasaland has been recorded by Stannus and Yorke,† an observation which has been confirmed more recently by the Royal Society's Commission.‡ The parasite has been isolated from a case of sleeping sickness originating in Portuguese East Africa,§, and it is accordingly probable that cases reported from the neighbouring portions of German East Africa are due to the same trypanosome. Finally, indigenous cases of sleeping sickness have recently been diagnosed in Southern Rhodesia, though we are not in a position to state the identity of the organism.

It will be seen, therefore, that *Trypanosoma rhodesiense* is widely spread over South Central Africa, and its distribution is in close association with that of *Glossina morsitans*, which has been shown to be the vector.

(*b*) CLINICAL FEATURES OF THE INFECTION

There is no essential difference between the clinical manifestations of the disease caused by *T. rhodesiense* and that due to *T. gambiense*, except possibly the greater virulence of the former.

In the earlier stages, enlargement of the lymphatic glands is commonly seen, and more or less irregular fever, with the accompanying symptoms of malaise, anorexia, headache, pains in the limbs, &c., is the rule. During the attacks of fever trypanosomes can readily be demonstrated in the peripheral blood, but as a rule are rather scanty. As the disease progresses oedemata of various parts of the body, more particularly the face, are common features, together with emaciation, loss of muscular power, ataxic gait, tremors, difficult speech, loss of memory, and other signs of nervous derangement. These symptoms gradually increase in intensity until finally the patient sinks into a condition of coma, and dies.

No exact data exist as to the duration of the disease in natives, but it would appear to be short. Many of the cases complained of

† Stannus and Yorke. Proc. Roy. Soc., B 84, 1911, p. 156.

‡ Royal Society Commission. Roy. Soc. Proc., B 85, 1912, p. 423.

§ Sleeping Sickness Diary. Nyasaland Protectorate.

no subjective symptoms of the disease when diagnosed, and presented very few objective signs, but in general they lived only a few months. Occasionally, however, a patient is more resistant, and one native definitely proved to be infected with *T. rhodesiense* is still alive and in a state of apparent good health a year later.

(*c*) IDENTITY OF TRYPANOSOME WITH *T. RHODESIENSE*

The essential characteristic of *T. rhodesiense*, Stephens and Fantham, is the occurrence of 'posterior nuclei,' i.e., amongst the short forms of the parasite, organisms in which the macronucleus is markedly displaced from the usual central to a decidedly posterior position are commonly seen in certain laboratory animals, more especially rats. This displacement may proceed to such a degree that the macronucleus may actually be situated behind the micronucleus at the aflagellar end of the trypanosome. As this peculiarity has not been observed in *T. gambiense*, the two strains of human trypanosomes can readily be distinguished. A further difference is noticed in the pathogenicity of the parasites, *T. rhodesiense* being much more virulent for all species of animals than *T. gambiense*.

At Nawalia and Ngoa, rats were subinoculated from sixteen cases of sleeping sickness, and in every instance the posterior nuclei were observed in stained preparations. The pathogenicity of these strains agreed closely with that of the Armstrong strain,* from which *T. rhodesiense* was originally described.

It will be understood, therefore, that in all our transmission experiments the strains of human trypanosomes utilised answered in all respects to the description of *T. rhodesiense*.

(*d*). TRANSMISSION OF THE TRYPANOSOME

Experiments to transmit the human parasite by *Glossina morsitans* were made at Nawalia in the Luangwa Valley, and at Ngoa on the Congo-Zambesi watershed. Nawalia, the site of an old station, is situated on the right bank of the Nyamadzi river,

* Bevan. Journal of Comparative Path. and Therapeutics, 1910, p. 160.
Yorke. Annals of Tropical Medicine and Parasitology, 1910, p. 351.

a tributary of the Luangwa, at an altitude of about 2,100 feet above the sea. Its position is approximately 12° 25′ S. and 32° 2′ E. Ngoa, 11° 40′ S. and 31° 30′ E. lies some 30 miles North of the station of Mpika, at a height of 4,400 feet above sea level.

The meteorological observations at Nawalia and Ngoa are synopsised in Tables 1 and 2.

Certain general conditions which attach to all the experiments may be mentioned.

The identity of the flies has been controlled both by direct examination of the external characters and by the preparation of the male genitalia, as recommended by Newstead,* so that we can state with some degree of confidence that we have been dealing only with *Glossina morsitans*, Westw.

All the experimental animals have been kept in fly-proof cages, the fronts of which were protected by a double layer of wire gauze, the inner composed of coarse, and the outer of mosquito meshing. The two layers were separated by a space of one inch in order to obviate the possibility of an animal being bitten while pressing its body against the front of the cage.

The feeding of the flies, and the changing into fresh bottles daily, was supervised personally, while the flies were kept in such a manner that they had no opportunity of obtaining food from other than the animals used in the actual experiments.

Experiments at Nawalia

A. With Laboratory-bred *Glossina morsitans*

Experiment 1

Commenced August 20th, 1911.

It is somewhat difficult to tabulate this experiment, owing to the fact that it was not started on a definite date with a definite number of flies. Between August 20th and September 29th twenty-six flies had hatched out, and each, as it did so, was given its first meal on an animal showing numerous parasites in the peripheral blood, so that on any given date the periods which had elapsed since the infecting feeds of the flies varied considerably. In Table 3 the main facts in connection with the flies are given.

* Newstead, R. Bull. Entomol. Research, Vol. II, Part 1, May, 1911.

FIG. 1. Camp at Nawalia, Luangwa Valley.

FIG. 2. Laboratory at Nawalia, Luangwa Valley.

C. Tinling & Co., Ltd., Imp.

Table 1.—Meteorological observations at Nawalia, N. Rhodesia, 12° 25′ S., 32° 2′ E. Altitude 2,100 feet (approximately).

1911-1912	External Shade Temperatures			Laboratory Temperatures			Relative humidity %	Rainfall inches	Days on which rain fell
	Mean	Absolute maximum	Absolute minimum	Mean	Absolute maximum	Absolute minimum			
June	67·2	89·0	44·3	—	—	—	48·6	0	—
July	68·7	91·0	44·6	67·4	82·2	51·8	45·7	0	—
August	73·3	96·8	51·2	71·2	85·5	57·8	35·8	0	—
September ...	77·5	103·3	54·0	71·5	92·5	61·5	31·5	0	—
October	86·1	107·6	62·3	84·5	99·5	71·2	31·8	0·26	2
November ...	87·1	107·8	67·9	84·6	96·0	74·5	41·1	1·61	8
December ...	82·3	101·7	67·8	79·6	91·5	71·0	69·1	8·54	20
January	80·6	101·1	67·0	78·4	87·1	72·8	77·7	14·97	16
February ...	79·2	96·0	66·1	77·1	84·9	71·0	73·8	5·55	16
March	79·0	97·6	61·0	72·0	86·8	67·5	62·5	5·10	6
April (to 9th)	79·5	97·9	59·8	77·2	87·7	61·9	58·0	0·01	1
								36·04	69

Table 2.—Meteorological observations at Ngoa, N. Rhodesia, 11° 40′ S., 31° 30′ E. Altitude 4450 feet (approximately).

1912	External Shade Temperatures			Laboratory Temperatures			Relative humidity %	Rainfall inches	Days on which rain fell
	Mean	Absolute maximum	Absolute minimum	Mean	Absolute maximum	Absolute minimum			
April (20th to 30th)	72·0	89·7	54·1	68·0	76·8	59·0	53·7	0	—
May	69·0	90·5	45·1	66·2	77·0	51·0	51·3	0	—
June	61·6	85·5	34·3	60·5	72·5	42·0	53·0	0	—
July	62·4	83·0	42·3	60·9	72·0	49·1	53·0	0·01	1
August	67·7	86·4	45·2	65·7	71·5	60·0	46·7	0	—
								0·01	1

Table 3.—Giving date of infecting meal, date of death, and duration of life after infecting meal.

No. of fly	Date of infecting meal	Date on which fly died	Duration of life from date of infection
A 1	20.8.11	22.9.11	33 days
A 2	21.8.11	20.10.11	60 ,,
A 3	25.8.11	6.9.11	12 ,,
A 4	26.8.11	23.10.11	58 ,,
A 5	27.8.11	28.10.11	62 ,,
A 6	27.8.11	17.10.11	51 ,,
A 7	28.8.11	11.10.11	44 ,,
A 8	29.8.11	11.11.11	74 ,,
A 9	30.8.11	12.9.11	13 ,,
A 10	31.8.11	20.10.11	50 ,,
A 11	31.8.11	12.9.11	12 ,,
A 12	3.9.11	27.9.11	24 ,,
A 13	3.9.11	5.9.11	2 ,,
A 14	5.9.11	28.9.11	23 ,,
A 15	6.9.11	25.10.11	49 ,,
A 16	7.9.11	4.11.11	58 ,,
A 17	8.9.11	3.10.11	25 ,,
A 18	8.9.11	14.10.11	36 ,,
A 19	9.9.11	19.10.11	40 ,,
A 20	9.9.11	13.9.11	4 ,,
A 21	13.9.11	23.10.11	40 ,,
A 22	16.9.11	14.11.11	59 ,,
A 23	17.9.11	27.10.11	40 ,,
A 24	25.9.11	3.11.11	39 ,,
A 25	26.9.11	29.9.11	3 ,,
A 26	26.9.11	29.10.11	33 ,,

Twenty-four hours after each fly had fed on an infected animal, it was afforded an opportunity of feeding on a clean monkey (No. 41), after forty-eight hours on a second (No. 42), and from the third day onwards on a third (No. 52). The schedule of feedings is given in Table 4.

TABLE 4.—Showing transmission of human trypanosomes by laboratory-bred *Glossina morsitans*.

Date	Animal	No. flies fed	Result	Remarks
Aug. 21—Sept. 18...	Monkey 41	5*	Negative	Flies fed 24 hours after infecting feed.
,, 22 ,, 28...	,, 42	14*	,,	Flies fed 48 hours after infecting feed.
,, 23 ,, 26...	,, 52	23	Infection	Flies fed 72 hours and onwards after infecting feed.
Sept. 27 and 28 ...	,, 68	5	Negative	(*a*) Infecting feed over 30 days before.
	,, 69	6	,,	(*b*) Infecting feed between 20 to 30 days before.
	,, 70	5	Infection	(*c*) Infecting feed less than 20 days before.
Sept. 29—Oct. 3 ...	,, 72	16	,,	All the flies fed.
Oct. 4	White rat 77	16	,,	All the flies fed.
,, 5	Monkey 68	14	Negative	Infected fly did not feed.
,, 6—9... ...	,, 58	16	Infection	All the flies fed.
,, 9	,, 61	1	,,	Infected fly, only, fed.
,, 11—12 ...	,, 68	14	,,	Infected fly fed on 13th and 14th as well.
,, 13—16 ...	,, 69	13	Negative	Infected fly did not feed.
,, 16—19 ...	,, 83	12	Infection	Infected fly commenced feeding on 16th, others on 17th.
,, 20—28 ...	,, 69	10	Negative	Infected fly did not feed.
,, 29—Nov. 11...	,, 56	4	,,	Infected fly did not feed.

* The remaining flies refused to feed.

From this table it will be seen that neither of the first two monkeys on which the flies were allowed to feed became infected, whereas No. 52 did so on the 27th September.

A reference to Table 3 will show that up to, and including the 26th September, twenty-three flies had fed on an infected animal more than three days previously, and had, accordingly,

been fed on Monkey No. 52. The three flies, A 24, 25 and 26, had never fed on this animal, and therefore had not to be considered in the attempt to isolate the infected fly. Moreover, six of the flies, A 1, 3, 9, 11, 13 and 20, had died prior to the 26th September, and of these, three proved to be negative throughout on examination. The other three, namely, A 3, 11 and 20, were found to show a heavy intestinal infection of trypanosomes. Fly A 3 died on September 6th, A 11 on September 12th, and A 20 on September 13th, while the monkey did not become infected until September 27th, much too long an incubation period for one of these flies to have been the infecting one. We have additional proof for the conclusion in that the abdominal contents (gut + salivary glands) of flies A 3 and A 20, on inoculation into monkeys, did not determine an infection.

On the 26th September there were, then, twenty flies with which to deal, amongst which was at least one infective fly. As stated above, three flies, A 24, 25 and 26, had never fed on Monkey No. 52, so that the inquiry was limited to seventeen, and this was further reduced to 15 by the death of flies A 12 and 14 on September 27th and 28th—both flies negative on examination. These were accordingly split up into three groups, based on the length of time which had elapsed since the date of the infecting feed, and each group was allowed to feed for two days on a clean monkey, Nos. 68, 69 and 70.

Group (*a*) Infecting meal over thirty days previously.
,, (*b*) ,, ,, between twenty and thirty days previously.
,, (*c*) ,, ,, less than twenty days previously.

Of the three monkeys, No. 70 was the only one to become infected, and the transmitting fly was thus located in Group (*c*), consisting of A 19, 21, 22 and 23.

While waiting to ascertain which of the three monkeys would become infected, all the flies were fed from September 29th to October 3rd on Monkey No. 72, and on October 4th on White Rat No. 77. Both of these animals became infected in due course.

On October 5th all the flies, with the exception of A 19, were re-fed on Monkey No. 68, and from the 6th to the 9th all were fed on Monkey No. 58, except on October 9th when fly A 19 alone,

was fed on Monkey No. 61. Of these animals, No. 68 did not become infected, while Nos. 58 and 61 did. The fly A 19 (♂) was thus proved to be the infecting one.

No particular interest attaches to the further experiments. From the table it will be seen that both those animals on which fly A 19 fed became infected (Nos. 68 and 83), whereas those on which it did not feed remained quite healthy (Nos. 69 and 56).

When it had been definitely ascertained which was the infecting fly, it was possible to determine fairly accurately the duration of the cycle of the trypanosomes in the insect. Fly A 19 had its infecting meal on September 9th, and Monkey No. 52, the first to become infected, showed trypanosomes in the peripheral blood on September 27th. On the 26th, therefore, the last day on which the flies were fed on this animal, a period of eighteen days had elapsed since A 19 fed on the infected animal. The average incubation period of the local strain of human trypanosomes in monkeys is four to five days, and subtracting this from the eighteen days, it is evident that the fly must have become infective in thirteen days.

This fly, A 19, lived for forty days from the date of the infecting meal, and between the time of becoming capable of transmitting the parasite and the date of death had fed on eight animals, all of which became infected.

The other flies were fed continuously from the date of the possible infecting meal to that of death, which occurred at varying periods from two to seventy-four days, but none of them became infective.

Experiment 2

Commenced November 14th, 1911, with sixteen laboratory-bred *Glossina morsitans*.

In this experiment the flies were infected directly on a case of human trypanosomiasis, each fly being allowed to feed on one occasion only. Ten fed on the 14th of November, when the patient showed three trypanosomes per field in the blood, and the remaining six on the 15th, when there was, on an average, one trypanosome to seven fields (Zeiss Oc. 4, Obj. D.D.). The subsequent meals were as shown in Table 5.

Table 5.—Showing transmission of human trypanosome by laboratory-bred *Glossina morsitans*.

Days after infecting feed	Animal	No. of flies fed	Result	Remarks
1 to 5	White rat 116	15	Negative	
6 to 10	,, 118	15	,,	
11 to 15	,, 124	15	Infection	
16 to 20	,, 116	13	,,	
21 to 22	,, 118	12	,,	
23 to 27	Monkey 137	6	Negative	Flies divided into two groups to separate the infective one.
23 to 27	,, 138	5*	Infection	
28 to 42	,, 148	Varied	Negative	

* One fly of this group (6) refused to feed, and died on the 26th day of the experiment.

Rat No. 124 became infected on December 4th, five days after the flies had fed last, and as the incubation period of the trypanosome in these animals, on an average, is five days, it seems probable that the fifteenth day was the one on which the infecting fly became capable of transmitting the parasite.

On the 7th of December (23rd day after infecting meal) the twelve flies then alive were divided into two groups in order to effect an isolation of the infective one, and were fed on Monkey Nos. 137 and 138, as indicated in the table. On December 12th, the fly numbered B 23 (♂) died, and on examination proved to be heavily infected throughout the alimentary canal, and in the salivary glands. No infection of the proboscis, however, was observed.

The other flies were fed on a clean monkey until the 42nd day, but without result.

Experiment 3.

Commenced December 29th, 1911, with twenty laboratory-bred flies.

These flies were infected directly from a patient in whose peripheral blood three trypanosomes per field (Zeiss Oc. 4,

Obj. D.D.) were seen. They were afterwards fed daily for sixty-five days on a series of healthy monkeys, none of which became infected. From the 67th—70th day of the experiment, the seven flies then alive were fed on a guinea-pig heavily infected with the human trypanosome, and were then fed for a further period of thirty days on a clean monkey. This did not become infected.

Experiment 4

Commenced January 12th, 1912, with twenty-three laboratory-bred flies.

These were fed for four days on a patient showing, on an average, one trypanosome to three fields in the peripheral blood, and afterwards on healthy monkeys, as indicated in Table 6.

TABLE 6.—Showing transmission of human trypanosome by laboratory-bred *Glossina morsitans*.

Day	Animal	No. of flies fed	Result	Remarks
0 to 3	Patient	23	—	
4	—	—	—	Flies starved.
5 to 8	Monkey 237	22	Negative	
9 to 12	,, 238	22	,,	
13 to 16	,, 240	22	,,	
17 to 20	,, 254	20	Infection	
21 to 23	,, 237	18	—	Died on 24th day.
24	,, 240	17	Infection	
25 to 29	,, 260	9	Negative	Flies divided into two groups.
	,, 261	9	Infection	
30 to 60	,, 272	16—0	Negative	Infected fly did not feed.

On February 20th, the 29th day of the experiment, the fly numbered D 18 died, and on dissection was found to show a massive intestinal infection of trypanosomes. Unfortunately, the fly had been dead for some hours before it was examined, and it was found impossible to dissect out the salivary glands. The whole abdominal contents, therefore (gut and glands) were crushed

up in normal saline solution and inoculated into a healthy monkey, which became infected five days later. The disease ran a typical course.

None of the other flies—dissected as they died—were found to harbour trypanosomes in the proboscis, gut, or salivary glands.

In this instance the time which elapsed from the date of the *first* infective meal until the date on which the fly became capable of transmitting the trypanosome (allowing five days for the incubation period in the monkey) was nineteen days.

B. By 'wild' *Glossina morsitans*

Experiment 5

Commenced on November 14th, 1911, with ninety-eight 'wild' flies.

Prior to infecting these flies with the human trypanosome, they were fed for three days, November 14th-16th, on a healthy monkey (No. 95), and for the next four days on a native fowl. The monkey never became infected. From the 21st to the 24th of November the insects then alive, fifty-seven in number, were fed on an infected monkey showing twenty to thirty trypanosomes per field in the peripheral blood, and were afterwards fed on healthy animals, as in Table 7.

Table 7.—Result of feeding 'wild' *Glossina morsitans* on clean monkeys, after a preliminary meal on an animal infected with the human trypanosome.

Days after first infecting feed	Animal	No. of flies fed	Result	Remarks
4 to 6	Monkey 125	48	—	Monkey died on 7th day.
7 to 9	,, 127	41	Negative	
10 to 13	,, 130	34	Infection	
14 to 16	,, 119	31	,,	
17 to 18	,, 140	7	Negative	Monkey died on 19th day.
17 to 23	,, 141	10	Infection	Flies divided into two groups to isolate infected one.
20 to 25	,, 144	4	,,	

This experiment was finished after the flies had fed on the 25th day, the flies being then killed and embedded.

The duration of the cycle of the parasites in the flies, in this instance, would appear to be slightly over eleven days. The first infecting meal was taken on November 21, and Monkey No. 130 showed parasites in the peripheral blood on December 7, a difference of sixteen days. As stated already, the incubation period of this trypanosome in monkeys is about five days, and by subtracting this from the sixteen days, we obtain eleven for the duration of the development cycle.

Experiment 6.

Commenced January 12, 1912, with forty-two freshly-caught flies.

After being fed for one day on a monkey infected with the human trypanosome, and showing numerous parasites in the peripheral blood, the flies were fed on a clean monkey for nine days. They were then starved for one day, and subsequently allowed to feed on clean monkeys and rats from the 11th to the 33rd day. None of these animals became infected. The flies were dissected as they died, and while trypanosomes were found in the gut and proboscis of several, in no instance was an infection of the salivary glands observed.

Experiment 7

Commenced January 12, 1912, with forty-two freshly-caught flies.

The details of this experiment are exactly similar to those of Experiment 6, with the exception that from the 1st to the 9th day the flies were fed on a native fowl instead of on a monkey. They were starved on the 10th day, as before, and afterwards fed on clean monkeys and rats from the 11th to the 38th day. None of these animals became infected. Trypanosomes were found in the proboscis and gut of several of the flies when dissected, but in no case were the salivary glands implicated.

Experiment 8

Commenced February 14th, 1912, with 104 freshly-caught flies.

On the 13th of February, the flies were fed on a healthy monkey

which did not become infected, thus excluding the possibility that they were already infected with the trypanosome. On the four succeeding days they were fed on a guinea-pig infected with the human trypanosome, and showing numerous parasites in the peripheral blood, and afterwards on clean monkeys as indicated in Table 8.

Table 8.—Showing transmission of the human trypanosome by freshly-caught *Glossina morsitans*.

Day	Animal		No. of flies fed	Result	Remarks
4					Flies starved.
5 to 10	Monkey	269	98	Negative	
11					Flies starved.
12	,,	269	64	,,	
13 to 27	,,	280	41	,,	Died on 28th day. Flies divided into two groups, A and B.
13 to 29	,,	281	47	Infection	
28 to 29	,,	286	33	Negative	Group A, only, fed.
30	,,	269	17	Infection	Group B, only, fed.
30 to 38	,,	300	10	Negative	A 1
30 to 40	,,	301	10	,,	A 2 — Flies of group A divided into 3 sub-groups, A 1, A 2, A 3.
30 to 40	,,	302	12	,,	A 3
31 to 33	,,	303	12	,,	B 1
31 to 37	,,	304	11	Infection	B 2 — Flies in group B divided into three sub-groups, B 1, B 2, and B 3.
31 to 34	,,	305	12	Negative	B 3
34 to 39	,,	310	11	,,	Sub-group B 1 fed.
35 to 52	,,	315	12	,,	Sub-group B 3 fed.
39 to 52	,,	300	15	,,	Sub-group A 1 and B 2 fed.
41 to 52	,,	301	28	,,	Sub-group A 2, A 3, and B 1 fed.

The insects were dissected as they died, but only in one, the infective fly, was an infection of the salivary glands observed, though in a considerable number an infection of the proboscis and gut was found.

The duration of the development cycle of the trypanosomes in the fly would appear to be twenty-five days in this experiment. The flies were fed for the *first* time on the infected guinea-pig on February 14th, and the first monkey became infected on March 15th, thirty days later. The average incubation period of the disease in monkeys is five days, so that the cycle took twenty-five days to complete.

Discussion of Results

In these transmission experiments, there are at least three sources of error which must be considered, (1) accidental infection of the experimental animals by other than the experimental flies, (2) hereditary transmission of trypanosomes from infected female flies to their progeny, and (3) natural infection in the experimental animals.

(1) With regard to the first of these, the conditions under which the experimental flies and animals were kept have been mentioned already, and it seems more than improbable that accidental infection would account for the unfailing regularity with which the animals became infected after the infective flies had fed on them. Moreover, in all our experiments, 644, such an occurrence as the unexpected infection of an animal has not been observed.

(2) The number of bred flies which we have been able to obtain has been too small to permit us to examine many of them prior to use in the experiments, but such as were, have been found uniformly free of infection. Stuhlmann,* Kleine,† and Bruce‡ with his colleagues have examined large numbers of bred flies belonging to the species *Glossina brevipalpis*, *Glossina morsitans*, and *Glossina palpalis*, and are unanimous in the opinion that hereditary transmission of trypanosomes does not occur amongst the tsetse flies.

(3) With reference to the third point, we have used in the course some 256 monkeys, and have never seen a naturally-occurring trypanosome infection in any of them. *Plasmodium kochi* and microfilaria have been observed, but beyond these, nothing.

* Stuhlmann. Arbeit aus d. Kaiser. Gesundheitsamte, Band XXVI, Heft 3, p. 374.

† Kleine. Deutsche med. Wochenschrift, No. 45, 1909.

‡ Bruce, Hamerton, Bateman and Mackie. Reports of S.S. Commission of the Royal Society, No. 11, pp. 122-125.

Experiment 2 was specially devised to obviate the possibility of error through the use of local monkeys (*Cercopithecus pygerythrus*). The flies, as they were obtained, were fed on healthy, imported rabbits which showed no signs of infection throughout; they were infected directly from the human host; and they were then fed on white rats. With the exception of Experiment 1, all the bred flies used in the transmission work were infected directly on the human host.

The trypanosomes transmitted by these flies were identical with the human one, both morphologically and in their animal reactions.

There are certain points in connection with the experiments which appear to be worthy of emphasis. The number of bred flies which has been used in each is strikingly small, very much more so than in any other similar work, of which the records are available. In the four experiments a total of eighty-five was employed, and of these three only became infective. A percentage of 3·5.

The time occupied by the trypanosomes in completing their cycle in the flies is also strikingly short, approximately two weeks (thirteen, fifteen, eleven, nineteen and twenty-five days).

It may be pointed out, however, that all our estimations of the latent periods of the trypanosomes in the flies represent the probable durations only. Although the average incubation period in monkeys is five days, this has been found to vary from three to eight days, and it is possible, therefore, that the cycle may have been slightly shorter, or longer, in any one instance.

Moreover, a further source of error is introduced in those experiments in which the flies were fed on an infected animal for more than a single day. It has yet to be determined whether only a definite percentage of flies are inherently capable of transmitting the disease, or whether *any* fly will do so provided that it has an opportunity of feeding on an infected animal at some particular time during its existence. If the latter alternative be correct, the peculiar factors governing their infectability have still to be ascertained. Assuming the first view to be correct, then the latent period of the trypanosomes in the flies must date from the *first* occasion on which the insects were fed on the infected animal, while, if the second be correct, the latent period may date from *any* of the meals on the infected animal.

So far as our results go, we have seen no indication of late infection in any of our flies, although some of them have lived as long as seventy-four days after the potentially infecting meal.*

All our results go to show that mechanical transmission of the trypanosomes does not occur, that is, if a period of twenty-four hours has elapsed since the infecting meal. We have not made any experiments to ascertain whether infection could be accomplished by interrupted feeding. This has been proved with various insects, but practically, would account for very few, if any, cases of the disease.

The infective flies have been found to retain the power of transmitting the parasites during life, and do not require to feed more than a single time on an animal in order to infect it, neither do they require, prior to becoming infected, to feed more than once on an animal suffering from trypanosomiasis.

With regard to Experiment No. 5, although only two flies were definitely proved to transmit the trypanosome, infection of the salivary glands was found, on dissection, in four others. As will be seen in Section V, this is strong presumptive evidence that these four were also infective.

Experiments at Ngoa

A. With Laboratory-bred *Glossina morsitans*

Experiment 9

Commenced on June 23rd, 1912, with nineteen laboratory-bred flies.

They were fed for five days on a heavily-infected guinea-pig, and afterwards for fifty-five days on a healthy monkey which did not become infected.

B. By 'Wild' *Glossina morsitans*

Experiment 10

Commenced on May 18th, 1912, with 116 'wild' *Glossina morsitans*, which had previously been shown to be non-infective by being fed on a healthy monkey. The flies were fed for four days on a heavily-infected guinea-pig and afterwards for sixty-seven days on three healthy monkeys, none of which became infected.

* See, however, page 209.

FIG. 1. Camp at Ngoa, Congo-Zambesi Watershed.

FIG. 2. Laboratory at Ngoa, Congo-Zambesi Watershed.

Experiment 11

Commenced on June 13th, 1912, with ninety 'wild' flies previously proved not to be transmitting the human trypanosome. They were allowed to feed for three days on a heavily-infected guinea-pig, and were afterwards allowed to feed on a healthy monkey until the 40th day of the experiment. This monkey did not become infected.

On the 41st day of the experiment the flies then alive, forty-two in number, were placed in an incubator, kept at a temperature of 85° F. Three flies were found to be infective eight days later.

Experiment 12

Commenced on June 14th, 1912, with 119 'wild' *Glossina morsitans*, proved to be non-infective. The flies were fed from the 1st to the 3rd day of the experiment on a heavily-infected guinea-pig, and afterwards to the 60th day on a healthy monkey, which did not become infected.

On the 61st day the thirty-eight remaining flies were placed in the incubator kept at 83° F., and were fed from the 61st-75th day on a clean monkey. The monkey died on the 76th day without becoming infected. However, on dissecting the flies, one was found to harbour trypanosomes in the gut and salivary glands, and the contents of these structures inoculated into healthy monkeys caused the animals to become infected with *T. rhodesiense*.

Experiment 13

Commenced on July 11th, 1912, with 176 'wild' *Glossina morsitans*, previously shown not to harbour the human trypanosome. These flies were fed for three days on a heavily-infected guinea-pig, and afterwards from the 5th—51st day on healthy monkeys, none of which became infected.

Experiment 14.

Commenced on July 24th, 1912, with 160 'wild' *Glossina morsitans*, previously proved to be non-infective. From the 1st—4th day they were fed on a heavily-infected guinea-pig, and from the 5th—36th on a healthy monkey, which did not become infected.

(*e*) INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE DEVELOPMENT OF THE TRYPANOSOME IN *GLOSSINA MORSITANS*

At Nawalia, eight transmission experiments were made, four with laboratory-bred, and four with 'wild' *Glossina morsitans*. At Ngoa, six experiments were carried out, five with 'wild' and one with bred flies. It is unfortunate that the bred flies in the latter series were not more numerous, but owing to the low temperature the majority of the flies did not emerge from the puparia, and many of those which did were malformed, and quickly died. In all the experiments in which 'wild' flies were used, however, the possibility that they were already infected with the trypanosome was excluded by first feeding them on healthy monkeys.

Synopses of the two series of experiments are given in Tables 9 and 10.

It will be seen from these tables that whereas, in the Luangwa Valley, *Trypanosoma rhodesiense* was successfully transmitted by *Glossina morsitans*, all efforts in this direction on the Congo-Zambesi watershed have been in vain. Of 330 flies used in the valley experiments, six, and probably ten, became infective. The larger figure is based on the number of salivary gland infections found in the flies. Our experience indicates that the implication of these structures is intimately connected with the ability of *Glossina morsitans* to transmit *Trypanosoma rhodesiense*, and that until they are invaded by the organisms the flies are non-infective. Salivary gland infections have been found in all the flies which were capable of transmitting the parasite. In Experiment No. 5 (Table 7) six flies were found to harbour trypanosomes in the glands, but of these only two were actually proved to transmit *Trypanosoma rhodesiense*. As in all other instances, it was shown conclusively that those flies in which trypanosomes were found in the salivary glands were infective, it may be concluded that the remaining four flies in this experiment were also capable of transmitting the parasite. Invasion of the salivary glands has not been observed except in those flies which were known to transmit *Trypanosoma rhodesiense*.

In the six plateau experiments, 680 *Glossina morsitans* were employed without a single fly becoming infective.

No.	Date on which started	Season	No. of flies used	Variety of flies used	Result	Duration of developmental cycle days	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity* %
1	20.8.11	Dry	26	Bred	Infection	13	75·1	90·0	60·5	35·0
2	14.11.11	Commence-ment of rains	16	,,	,,	15	83·5	93·5	74·5	44·8
3	14.11.11		57	Wild	,,	11	83·8	93·5	74·5	44·8
4	29.12.11	Rainy	20	Bred	Negative	—	78·2	87·1	72·8	74·5
5	12.1.12	Rainy	42	Wild	,,	—	78·0	85·7	72·8	74·2
6	12.1.12	,,	42	,,	,,	—	78·0	85·7	72·8	74·2
7	12.1.12	,,	23	Bred	Infection	19	77·9	85·7	72·8	75·4
8	13.2.12	,,	104	Wild	,,	25	77·3	86·0	71·0	66·5

* In the cases of the unsuccessful experiments, the mean temperature and the relative humidity have been calculated for the first 30 days only.

TABLE 10.—Synopsis of transmission experiments with *T. rhodesiense* and *Glossina morsitans*, carried out at Ngoa, Congo-Zambesi watershed.

No.	Date on which started	Season	No. of flies used	Variety of flies used	Result	Duration of developmental cycle	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity %
1	18.5.12	Dry	116	Wild	Negative	—	62·7	74·5	50·5	52·0
2	13.6.12	,,	90	,,	,,	—	59·1	72·4	42·0	52·0
3	14.6.12	,,	119	,,	,,	—	59·1	72·4	42·0	52·0
4	20.6.12	,,	19	Bred	,,	—	60·2	71·8	42·0	52·0
5	11.7.12	,,	176	Wild	,,	—	62·0	72·0	49·1	50·0
6	24.7.12	,,	160	,,	,,	—	64·7	76·1	51·0	47·0

The explanation of these apparently contradictory results is at first sight not very obvious, more particularly in view of the fact that even on the plateau 'wild' *Glossina morsitans* capable of infecting healthy monkeys with *Trypanosoma rhodesiense* were occasionally encountered. If the climatic conditions under which the valley experiments were carried out be compared with those obtaining during the plateau experiments, it will be seen at once that the most striking difference is one of temperature. As a rule, the temperature during the former series of experiments was roughly from 15-20° F. higher than during the latter series.

With a view to ascertaining the influence, if any, exerted by temperature on the developmental cycle of *Trypanosoma rhodesiense* in the tsetse fly, a further series of experiments were performed on the plateau, in which, by means of an incubator, the flies were kept at a temperature approximating to that of the valley at the most favourable season.

In the first two experiments 'wild' flies were used. No water was placed in the incubator, and the warm dry air was found to have a very deleterious effect on the insects. Within the first seven days, twenty-five of the sixty-one flies with which Experiment No. 1 was commenced, and fifty-three of the seventy-two in Experiment No. 2 had died.

Notwithstanding the small number alive at the end of the second week, two infective flies were obtained in the first experiment and one in the second. In the third experiment laboratory-bred flies were employed instead of 'wild' ones. Attention was drawn to the fact that the low temperatures obtaining at Ngoa in the cold season were very unfavourable to the pupation of *Glossina morsitans;* in fact, so slow was the process that in spite of the large number of pupae at our disposal, we were unable to procure sufficient flies for experimental purposes. The difficulty was all the greater as many of those which did emerge were malformed, and quickly died. In order to obtain a sufficient number of bred flies for this experiment we resorted to the expedient of placing the pupae, some of which had been deposited over two months previously, in the incubator (85° F.). Within three or four days a large number of flies were procured.

The experiment was commenced on August 8th with thirty bred

flies, to which were added twelve on the 9th, eleven on the 10th, and three on the 11th. These groups were fed for four, three, two and one days, respectively, on a heavily-infected guinea-pig and afterwards on a healthy monkey. Parasites were found in the blood of the animal on which the flies of the fourth group were fed on the 18th after the insects fed on the guinea-pig, so that, allowing five days for the incubation of the disease in the monkey, the fly was infective on the 13th day. The monkey on which the flies in Group 1 were fed became infected on the 26th day of the experiment, so that the duration of the developmental cycle of the parasite in the fly would be twenty-one days. As we cannot be certain that the infective fly fed on the infected guinea-pig on each of the four days, the latent period in the insect may be anything from seventeen to twenty-one days. The animals on which Groups 2 and 3 were fed did not become infected.

It will be seen from Table 11 that the total number of flies used in the incubator experiments was 189—133 'wild' and 56 'bred.' Of the 'wild' flies, three became infective, and of the 'bred' two, i.e., 2·6 % of the total number used. This figure is, however, hardly a fair estimate, as of the 133 'wild' flies only seventy-eight were alive at the end of the first seven days of the experiment. This heavy mortality was probably due to the sudden change from the cold external air to the warm, dry atmosphere of the incubator. As a general rule, in our transmission experiments it was found that about 10 % of the flies died during the first week. This was approximately the case in the incubator experiment in which bred flies were used, as, owing to the fact that the insects were hatched out in the incubator they were not subjected to any sudden change of atmospheric conditions. We consider, therefore, that had the mortality of the flies in these two experiments been the customary 10 % instead of over 40 %, the proportion of infective flies would be three of ninety instead of three of 133, or 3·3 % instead of 2·2 %. This figure, 3·3, approximates closely to that obtained in the incubator experiment in which bred flies were used, namely, 3·5, and also to that obtained in the valley experiments, 3·5.

The results of these three series of experiments, viz., those carried out at Nawalia at laboratory temperatures 75°-84° F.,

TABLE 11.—Synopsis of experiments to transmit *T. rhodesiense* at Ngoa, Congo-Zambesi Watershed, by means of *Glossina morsitans* kept in incubator.

No.	Date on which started	Season	No. of flies used	Variety of flies used	Result	Duration of developmental cycle days	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity %
1	30.6.12	Dry	61	Wild	Infection	14	80·6	87·8	74·5	36·0
2	1.7.12	,,	72	,,	,,	13	80·6	87·8	74·5	36·0
3	8.8.12	,,	56	Bred	,,	13 and 21	82·6	89·2	77·0	72·4

those made in the laboratory at Ngoa at from 59°-65° F., and the incubator experiments at Ngoa 80·6°-82·6° F., show in a most conclusive manner that comparatively high temperatures, 75°-85° F., are necessary for the completion of the developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans*.

In addition to temperature, there is another factor in the climatic conditions which might possibly influence the developmental cycle of the trypanosome in *Glossina morsitans*. We refer to the relative humidity of the atmosphere. At the most favourable season of the year in the Luangwa Valley for transmission experiments, and also in the case of the first two carried out in the incubator, the relative humidity was extremely low. In order to decide the point, the relative humidity of the atmosphere in the incubator in the bred fly experiment described above was kept at from 70-72·5%. As the incubation period of the parasite in the flies (twelve and seventeen to twenty-one days respectively) and also the percentage of infective flies obtained (3·5 %) were approximately the same as those in other experiments in which the relative humidity was very low, we can only conclude that this factor does not exert any appreciable influence on the developmental cycle of *T. rhodesiense* in *Glossina morsitans*.

The following experiments were devised with a view to ascertaining more definitely the influence of temperature on the development of the parasite.

Experiment 15

Two batches of 'wild' *Glossina morsitans* (Batch A consisting of 95 and Batch B of 119) in which the possibility of the presence of an infective fly had previously been excluded by feeding the insects on clean monkeys, were fed for three consecutive days on a guinea-pig infected with *T. rhodesiense*. After being starved for a day, each batch was fed on a healthy monkey until the 40th day after the first feed on the infected animal. Neither of the monkeys became infected. Batch A, in which there were forty-two flies still alive, was placed in the incubator, whilst Batch B, in which there were now fifty-eight flies, was kept at laboratory temperature. The sudden change from the laboratory to the warm, dry air of the incubator proved very fatal to the flies

in Batch A, and on the 43rd day only six were alive. From the 41st to the 47th day the flies in this batch were fed on a monkey, and from the 48th day on a rat. The rat became infected on the 53rd day, so that, allowing five days for the incubation of the disease in the animal, Batch A contained an infective fly on the 48th day after the first feed on the infected guinea-pig, and eight days after being placed in the incubator. As the monkey died on the 47th day, we are unable to state whether the fly became infective before the 48th day. The four flies still alive on the 53rd day were fed on four clean rats, and three of these became infected. The monkey on which Batch B was fed was still negative at the end of sixty days, when there were thirty-eight flies alive.

Experiment 16

This is really a continuation of the former experiment. The thirty-eight flies in Batch B were placed in the incubator on the 61st day after the first feed on the infected guinea-pig, and were fed from the 61st-75th day on a healthy monkey (No. 443). Unfortunately the animal died on the 76th day, so that we were unable to determine with certainty whether any of the flies became infective. All the flies were dissected as they died, and one was found to harbour trypanosomes in the gut and salivary glands. Animals inoculated with the contents of these structures became infected with *T. rhodesiense*. As in our experience all flies in which salivary infection was observed were capable of infecting animals with the human trypanosome, we may assume that had the monkey (No. 443) lived a few days longer it would have been found to be infected.

In Experiment 15 the relative humidity of the air in the incubator was very low (36 %), while in Experiment 16 the relative humidity was comparatively high (72 %). In addition to confirming the view that a relatively high temperature is essential to the completion of the developmental cycle of *T. rhodesiense* in *Glossina morsitans*, and that the relative humidity of the atmosphere is not an important factor, these experiments afford more definite information. It is apparent that the earlier stages of the development of the parasite in the fly can occur at comparatively low temperatures (60° F.), and that trypanosomes can persist in this

Table 12.—Synopsis of experiments showing effect of raising the temperature on the development of *T. rhodesiense* in *Glossina morsitans*.

No.	Date started	Season	No. of flies used	Variety of flies	Laboratory Conditions				Date on which flies were put in the incubator	No. of flies alive at the time they were first put in incubator	Incubator Conditions				Remarks
					Mean temp.	Absol. max.	Absol. min.	Rel. hum. %			Mean temp.	Absol. max.	Absol. min.	Rel. hum.	
15	13.6.12	Dry	90	Wild	59·1	72·4	42·0	52·0	24.7.12 (41st day)	42	85·0	90·5	77·0	36·0	Three flies became infective on 48th day.
16	14.6.12	,,	119	,,	59·1	72·4	42·0	52·0	14.8.12 (61st day)	38	83·0	91·0	77·0	72·0	One fly became infective about the 72nd day.

stage for at least sixty days. It is obvious that the developmental cycle of the parasite is not complete, since the flies are non-infective, and inoculation of the gut contents into susceptible animals is followed by negative results. For the completion of the cycle it is necessary for the temperature to which the flies are subjected to be raised to a considerable extent (75°-85° F.).

It is interesting to note that the flies in Batch A (Experiment 15) became infective eight days, possibly less, after being placed in the incubator. This is three days less than the shortest incubation period observed in any of our successful transmission experiments, a fact which supports the view that the developmental cycle of the parasite in the fly had proceeded to a certain point at laboratory temperature (60° F.) before the insects were subjected to the higher temperature (80° F.) of the incubator.

The fact that an occasional infective 'wild' fly was encountered on the plateau during a period (May, June and July) when attempts to transmit in the laboratory were invariably unsuccessful requires some explanation. A possible solution may be that the flies in question were infected during the warmer season of the year and had survived into the cold season.

If the results obtained by feeding freshly-caught flies on healthy monkeys in the valley are compared with those from flies caught on the plateau, a marked difference in the number of infections resulting is apparent. In the Luangwa Valley, 3,202 flies were fed in twenty-nine batches, and *Trypanosoma rhodesiense* was isolated in six of the experiments, giving a ratio of 1 infective fly to 534, whereas on the Congo-Zambesi watershed, 5,250 freshly-caught *Glossina morsitans* were fed in groups on forty-one monkeys, with four positive results—1 infective fly to 1,312. As tsetse flies and game are about equally numerous at Nawalia and Ngoa, and as the disease was presumably introduced into the two localities, which are less than seventy miles apart, about the same time, it appears to us that the only essential difference which can account for the fact that the percentage of infective 'wild' flies at Nawalia is two and a half times as great as at Ngoa is the difference in the climatic conditions. It will be seen from Tables 1 and 2 that the temperatures experienced on the Congo-Zambesi watershed during May, June and July, are very much lower than those at Nawalia

FIG. 1. Feeding *Glossina morsitans* on experimental animals.

FIG. 2. Feeding *Glossina morsitans* on experimental animals.

from September to March. It was during the months named that our experiments were carried out at the two places.

Finally, it might be mentioned that the percentage of infective 'wild' flies caught in the valley was greater in the hot than in the cold season. This point is illustrated in Table 13.

TABLE 13.—Percentage of *Glossina morsitans* found infected with *Trypanosoma rhodesiense* at Nawalia at different seasons of the year

1911-1912	Mean external shade temp.	No. of flies fed	No. infective with *T. rhodesiense*	Ratio of infective to non-infective flies
June	67·2	18	0	
July	68·7	385	0	0 : 790
August ...	73·3	193	0	
September ...	77·5	194	0	
October ...	86·1	—	—	
November ...	87·1	270	1	
December ...	82·3	205	0	1 : 338
January ...	80·6	538	2	
February ...	79·2	104	0	
March ...	79·0	823	2	1 : 466
April (to 9th)	79·5	472	1	

The facts brought to light in this investigation afford a satisfactory explanation of some phenomena which have hitherto appeared to be contradictory. Kleine* was unable to transmit *T. gambiense* by *Glossina morsitans* on the Victoria Nyanza (altitude 3,700 feet), whereas Taute† was successful on Lake Tanganyika at a lower altitude (2,680 feet). Although we were unable to transmit *T. rhodesiense* experimentally on the Congo-Zambesi watershed in the cold season, nevertheless we found that a certain percentage of 'wild' flies was infective. This apparent

* Kleine. Deutsche med. Wochenschrift, No. 45, 1909.

† Taute. Reviewed in Bull. S.S. Bureau, No. 31, Nov., 1911.

discrepancy may be explained on the assumption that the flies became infective during the warm season and that a certain number survived into the colder season of the year.

(*f*) THE RESERVOIR OF THE TRYPANOSOME

The possibility that game might act as a reservoir of infection of sleeping sickness areas has been recognised almost since the inception of work on the disease, but up to the present it would appear that the trypanosomes have never been demonstrated in such animals under natural conditions. In Uganda, Bruce, Hamerton and Bateman* have proved that certain species of buck, notably waterbuck, bushbuck, and reedbuck, can readily be infected with *Trypanosoma gambiense* by allowing infected *Glossina palpalis* to feed on them, and that healthy flies, in turn, may be infected from game harbouring parasites in their blood. They were unable, however, to examine a sufficiently large number of head to ascertain whether a natural infection was present.

The importance of the question is obvious, and the results of our investigations on the point afford a striking commentary on the potential danger involved in the infection of the game.

The Luangwa Valley is particularly rich in a widely-varied fauna, and owing to the fact that in the dry season the great bulk of the game tends to collect in the vicinity of the few permanent streams, it has been comparatively simple to shoot buck for the purposes of experimentation. At Ngoa, on the Congo-Zambesi watershed, game is plentiful at certain seasons of the year.

Trypanosomes indistinguishable from *T. rhodesiense* were isolated from buck in both these localities, and, in addition, from one native dog living in a village some fifty miles to the East of Nawalia.

In the Luangwa Valley 127 head of game were examined, and sub-inoculations into healthy monkeys and rats were made from 56. In this manner the human parasite was recovered from the following animals:—

4 Waterbuck,
1 Hartebeest,
2 Mpala,
1 Bushbuck,
1 Warthog.

* Bruce, Hamerton and Bateman. Proc. Roy. Soc., B, Vol. 83, 1911.

On the plateau 124 head were examined, and sub-inoculations were made from sixty. The trypanosome was isolated from two waterbuck only.

There is thus a marked difference in the percentage of game infected with *T. rhodesiense* in the two districts, the parasite being five times as frequent in the valley as in the plateau game.

As the game was shot without discrimination, the figures are probably a fair indication of the proportion of the total game infected in the two areas.

TABLE 14.—Percentage of game infected with *T. rhodesiense*.

NAWALIA (Luangwa Valley)			NGOA (Congo-Zambesi Watershed)		
No. inoculations made	No. infected	%	No. inoculations made	No. infected	%
56	9	16	60	2	3·3

As stated above, the trypanosome was isolated from one native dog only of thirty-five domestic animals examined—cattle, goats and dogs.

Our experience indicates that big game is much the most important reservoir of the infection. During our sojourn in the country some 256 monkeys, 142 wild rats, and 15 wild mice were examined, with negative results. In all, therefore, 698 animals were examined.

Apart from the bigger species of game, most of the smaller wild animals are nocturnal in their habits, and it seems unlikely that many of them would be exposed to infection.

(*g*) OCCURRENCE OF THE TRYPANOSOME IN *GLOSSINA MORSITANS* IN NATURE

Series of experiments were carried out in the Luangwa Valley and on the plateau to determine the species of trypanosome transmitted, in nature, by *Glossina morsitans*.

A number of fly-boys were sent out from time to time to capture and bring into the laboratory 'wild' tsetse flies, which were allowed to feed on healthy monkeys. Full details of these experiments will be found in a later section of this report.

At Nawalia (Luangwa Valley) the human trypanosome was

isolated in six of twenty-nine experiments, in which 3,202 tsetse flies were used. Possibly it was present in a seventh, but as the monkey died on the day after becoming infected, no definite statement can be made other than that the incubation period of the disease was the same as that in known *T. rhodesiense* infections.

At Ngoa (Congo-Zambesi watershed) 5,250 freshly-caught *Glossina morsitans* were fed in forty-two batches on healthy monkeys, and the trypanosome was isolated on four occasions.

The ratio of infective to non-infective flies in the two localities, assuming that only one was capable of transmitting the virus in each instance, is, therefore,

At Nawalia,	At Ngoa,
1 : 534.	1 : 1,312.

No definite comparison can be made between these figures, as the experiments were not carried out under identical conditions. Those at Nawalia were made during both the dry and wet seasons, while those at Ngoa were carried out during the height of the winter at a time when it was impossible to transmit the human trypanosome in the laboratory. However, the difference is so marked that it may safely be concluded that the plateau flies are infective, in nature, to a much smaller extent, than those in the Luangwa Valley.

In one of these experiments the actual infective fly was isolated.

Experiment 17

Commenced October 30th, 1911, with sixty freshly-caught flies, to which were added twenty-two additional ones on the next day. The flies were fed as indicated in Table 15.

On November 13th, the flies still alive, thirteen in number, were killed and embedded. In the sections, numerous parasites were found in the gut and salivary gland of only one of them.

Table 15.—Showing the transmission of the human trypanosome by naturally-infected *Glossina morsitans*.

Date	Animal	No. of flies fed	Result	Remarks
Oct. 30—Nov. 4 ...	Monkey 96	60-22	Infection	
Nov. 6	,, 105	29	,,	
Nov. 7—10 ...	,, 108	19	,,	
Nov. 11—12 ...	,, 113	7	Negative	Flies divided into two groups to isolate the infected fly.
Nov. 11—12 ...	,, 114	6	Infection	

(*b*) IDENTITY OF 'GAME' AND 'FLY' STRAINS WITH THE 'HUMAN' STRAIN OF *T. RHODESIENSE*

The conclusion that one of the trypanosomes isolated from game and from naturally-infected tsetse flies is identical with *T. rhodesiense* has been based on a careful study of the morphology and pathogenicity of the three strains in question.

1. *Morphology* (Pl. XVIII)

In fresh preparations, all three strains show the same mixture of short, slowly-moving, and long, active forms, the relative numbers of which vary in the peripheral blood of any animal from day to day.

In stained preparations, it is sufficient to say that it is impossible to distinguish any one of the three strains from the others. Short forms in which the macronucleus lies actually posterior to the blepharoplast have been observed in each of the three strains.

The measurements of the three strains also show an extremely close agreement. Eleven hundred individuals of each have been measured, and the results are given in Tables 16, 17 and 18. The total number of parasites drawn from each variety of laboratory animal is the same in the case of each strain, and only twenty-five have been measured from any one preparation, as it has been found that the average length varies within wide limits, from day to day, in any given animal.

We are of the opinion that the measurement of twenty-five individuals from one preparation gives a fair estimate of the average length of the trypanosome present in the particular blood film, and that the error is less if twenty-five parasites are measured on each of forty-four different days than if, for example, 100 are measured on each of eleven different days.

In measuring the parasites the following technique was adopted:—Thin blood smears, dried in the air, were fixed in absolute alcohol and stained with Giemsa's solution. The trypanosomes were then outlined at a magnification of 2,000 diameters with the aid of an Abbé camera lucida, and the length along the middle line of the body measured by the tangent method described by Stephens and Fantham.*

* Roy. Soc. Proc., B, Vol. 85, p. 223 (1912).

TABLE 16.—Giving details of measurement of 1,100 individuals of the 'human' strain.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Monkey	5	6	25	21·03	27·75	15·5
,,	5	11	25	19·5	26·19	13·27
,,	6	8	25	19·41	28·0	13·5
,,	6	15	25	26·3	31·5	19·67
,,	20	9	25	22·3	30·3	17·1
,,	20	13	25	19·97	26·25	13·25
,,	25	9	25	21·28	28·25	18·0
,,	25	13	25	19·57	29·75	15·25
,,	33	8	25	24·2	28·75	16·75
,,	33	9	25	20·59	29·75	15·29
,,	87	11	25	22·81	31·5	18·25
,,	87	21	25	22·41	29·25	18·5
,,	87	22	25	19·95	27·0	17·0
Dog	244	6	25	22·26	29·25	18·75
,,	244	8	25	20·16	24·5	17·5
,,	244	13	25	21·72	31·25	18·25
,,	244	14	25	19·7	22·5	17·25
Rabbit	13	4	25	23·5	30·5	14·5
,,	13	22	25	18·11	24·75	15·5
,,	A	24	25	19·52	39·25	14·5
,,	86	13	25	21·91	29·0	16·75
Guinea-pig	14	14	25	21·09	30·25	17·5
,,	14	20	25	22·03	31·75	16·0
,,	14	22	25	22·21	33·25	14·5
,,	139	25	25	20·66	27·25	15·75
,,	139	36	25	18·2	26·5	14·0
,,	139	52	25	18·4	28·0	13·75
Rat	15	22	25	20·03	25·5	15·75
,,	15	26	25	21·08	28·25	16·75
,,	16	15	25	22·98	33·25	15·70
,,	183	12	25	22·44	30·75	18·75
,,	184	12	25	22·12	31·25	18·0
,,	184	14	25	19·64	31·0	14·25
,,	184	20	25	20·17	27·5	17·0
,,	184	28	25	20·59	30·0	16·75
,,	208	10	25	19·32	23·25	16·75
,,	208	20	25	19·69	24·5	17·0
,,	212	6	25	23·33	31·0	18·75
,,	212	7	25	20·88	32·5	15·5
,,	212	16	25	18·66	22·5	13·75
Mouse	27	12	25	20·95	26·75	18·5
,,	28	6	25	19·94	23·0	17·25
,,	91	6	25	23·94	27·25	18·5
,,	91	10	25	28·65	33·0	21·5
			1100	21·25	39·0	13·25

TABLE 17.—Giving details of measurement of 1100 individuals of 'game' strain.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Monkey 71	7	25	24·79	32·9	17·0
,, 71	9	25	19·84	23·8	15·3
,, 99	38	25	26·36	34·25	19·0
,, 120	8	25	20·02	23·5	18·0
,, 120	11	25	21·9	29·25	17·25
,, 120	13	25	17·4	20·0	15·0
,, 130	8	25	25·97	35·5	19·0
,, 130	11	25	22·05	30·5	16·25
,, 199	5	25	22·47	25·75	15·75
,, 199	7	25	23·6	32·25	16·75
,, 201	7	25	23·4	31·0	17·75
,, 201	8	25	21·62	25·5	17·5
,, 201	9	25	19·58	21·75	17·25
Dog, native	?	25	19·1	26·0	15·5
,, 262	5	25	21·69	25·75	18·5
,, 262	7	25	19·13	23·5	13·5
,, 262	11	25	18·34	22·5	16·25
Rabbit 79	11	25	20·02	29·0	15·2
,, 249	9	25	16·18	19·5	13·75
,, 249	13	25	22·29	32·0	15·25
,, 249	13	25	20·91	28·5	15·75
Guinea-pig 251	10	25	20·87	33·25	15·25
,, 251	11	25	22·87	34·5	15·75
,, 251	13	25	23·11	33·75	15·0
,, 251	15	25	23·5	32·25	14·75
,, 251	17	25	24·09	34·25	13·75
,, 251	21	25	21·67	29·75	14·5
Rat 81	14	25	21·05	31·5	16·0
,, 128	20	25	20·25	21·75	17·5
,, 128	22	25	20·3	23·75	16·0
,, 129	?	25	20·9	28·0	16·25
,, 157	21	25	25·65	30·5	14·5
,, 157	42	25	19·27	21·5	16·75
,, 157	49	25	22·8	32·5	16·25
,, 195	26	25	21·8	35·0	16·5
,, 195	36	25	19·0	24·5	17·0
,, 213	17	25	17·38	19·0	14·5
,, 213	26	25	22·31	34·25	17·5
,, 221	7	25	18·91	23·0	16·5
,, 221	14	25	21·91	35·5	11·75
Mouse 176	9	25	20·13	26·5	17·5
,, 176	14	25	20·99	26·5	16·75
,, 178	6	25	22·89	29·5	17.25
,, 178	7	25	21·6	27·0	16·5
		1100	21·38	35·5	11·75

Table 18.—Giving details of measurement of 1100 individuals of 'fly' strain.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Monkey	96	7	25	25·7	32·0	16·0
,,	96	8	25	24·8	33·5	16·5
,,	96	9	25	25·6	36·25	16·0
,,	96	10	25	23·3	30·75	15·75
,,	96	11	25	22·6	31·0	15·25
,,	96	14	25	20·3	23·5	16·5
,,	114	27	25	22·0	28·0	16·25
,,	114	32	25	20·9	25·25	18·0
,,	114	41	25	20·8	30·75	15·25
,,	210	8	25	24·66	30·56	17·5
,,	210	10	25	20·29	23·25	18·25
,,	217	9	25	26·03	30·75	22·0
,,	316	9	25	24·69	32·25	17·5
Dog	235	5	25	26·7	33·0	19·0
,,	235	7	25	21·4	28·0	19·0
,,	235	9	25	20·0	28·0	18·25
,,	235	13	25	20·0	21·25	18·5
Rabbit	245	7	25	23·5	29·5	16·5
,,	245	8	25	20·0	28·0	14·5
,,	245	9	25	18·75	27·75	16·25
,,	245	13	25	22·84	30·0	17·0
Guinea-pig	246	13	25	19·87	23·5	16·7
,,	246	15	25	20·88	26·0	16·25
,,	246	18	25	17·63	21·5	13·0
,,	246	19	29	19·0	27·25	16·5
,,	246	20	25	18·95	25·25	14·25
,,	246	21	25	21·0	27·5	15·25
Rat	103	4	25	24·1	30·0	17·0
,,	103	5	25	20·3	30·0	16·5
,,	103	8	25	18·8	30·75	14·5
,,	218	6	25	19·47	24·75	16·5
,,	218	9	25	19·3	29·0	14·5
,,	218	14	25	20·1	22·5	17·0
,,	218	16	25	19·41	26·75	16·0
,,	218	18	25	22·0	30·5	18·0
,,	229	6	25	24·55	29·5	19·0
,,	229	8	25	21·09	29·25	17·0
,,	229	9	25	19·5	21·75	17·5
,,	229	13	25	22·3	29·5	18·5
,,	229	15	25	20·31	22·75	17·0
Mouse	247	4	25	23·1	29·5	19·5
,,	247	6	25	23·66	29·5	19·25
,,	247	9	25	22·6	34·0	18·75
,,	247	14	25	20·91	25·25	17·25
			1100	21·67	36·25	13·0

TABLE 19.—Comparison of the measurements of the 'human,' 'game,' and 'fly' strains.

Strain	Length in microns		
	Average	Maximum	Minimum
'Human'	21·25	39·0	13·25
'Game'	21·38	35·5	11·75
'Fly'	21·67	36·25	13·0

The similarity in the measurements is, perhaps, best appreciated by a glance at the curves obtained by plotting out the distribution of the various lengths of the parasites, expressed in percentages of the total numbers measured (see Chart I).

A comparison of the percentages of 'short and stumpy,' 'intermediate' and 'long' forms is also of interest.

TABLE 20.—Comparison of percentages of 'short and stumpy,' 'intermediate,' and 'long' forms of the 'human,' 'game,' and 'fly' strains.

Strain	Short and stumpy forms 11-21μ	Intermediate forms 22-24μ	Long forms 25-39μ
'Human'	64·78	15·98	19·14
'Game'	62·87	15·34	21·56
'Fly'	58·68	18·81	22·41

2. *Pathogenicity*

The pathogenicity of the three strains is synopsised in Table 21, p. 223. A glance at this table will show how remarkably the three strains agree in this respect.

CHART I. Comparison of curves of the 'human,' 'game,' and 'fly' strains by plotting out the distribution of the various lengths of the parasites, expressed in percentages of the total numbers measured (1,100 of each strain).

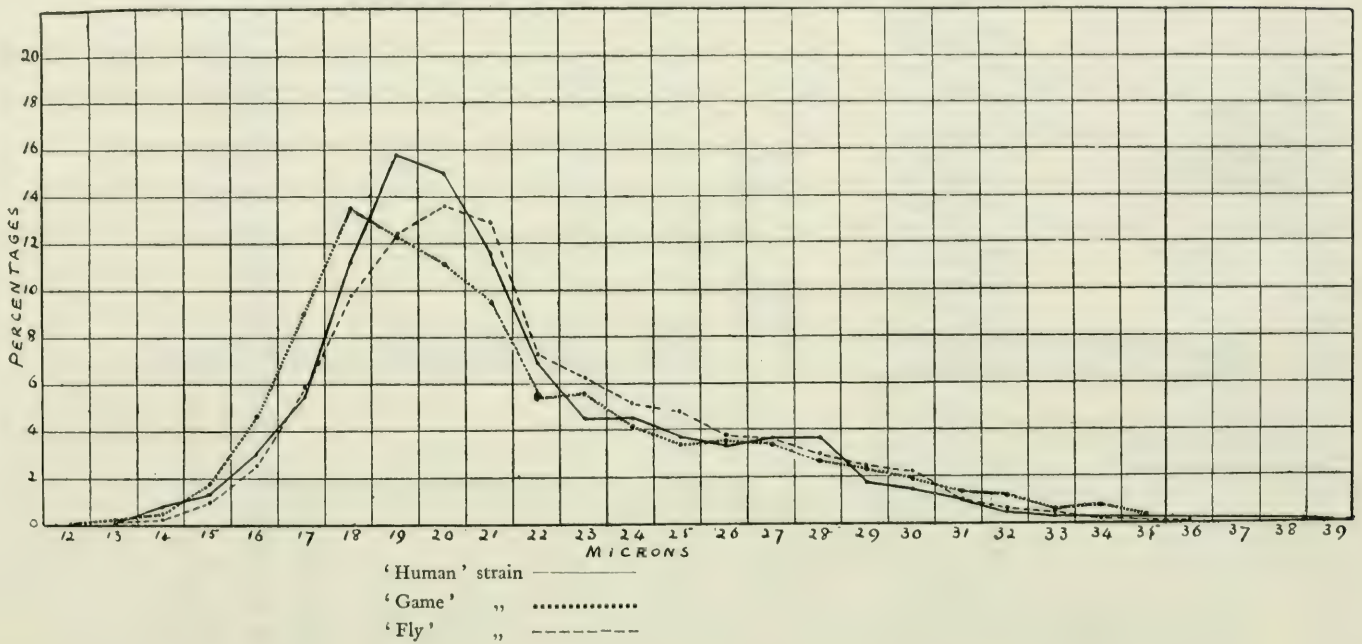

TABLE 21.—Comparison of the pathogenicity of the 'human,' 'game' and 'fly' strains of *T. rhodesiense*.

Animal	'HUMAN' STRAIN			'GAME' STRAIN			'FLY' STRAIN		
	No. used	Incubation days	Duration days	No. used	Incubation days	Duration days	No. used	Incubation days	Duration days
Monkey	47	3—9 Average 5	6—42 Average 18	14	4—11 Average 6	7—63 Average 17	12	4—11 Average 6	7—60 Average 24
Ox	1	7	209	1	7	210	1	7	186
Dog	1	5	26	1	5	25	1	5	26
Rabbit	5	4—6 Average 5	15—62 Average 31	5	4—8 Average 6	14—32 Average 26	5	4—7 Average 5	19—39 Average 29
Guinea-pig ...	6	6—19 Average 11½	48—119 Average 76	5	4—17 Average 11	53—100 Average 72	4	11—17 Average 14	47—127 Average 74 (1 alive after 55 days)
Rat	41	2—8 Average 5	7—83 Average 27½	15	3—8 Average 5	11—87 Average 35	10	2—7 Average 4	9—58 Average 34
Mouse	4	4	15—63 Average 32	2	4—5 Average 4½	48—51 Average 44½	1	4	92
Genet	1	6	76						

SUMMARY

1. The human trypanosome (*T. rhodesiense*) is distributed widely throughout South Central Africa.

2. There is no essential difference between the clinical manifestations of the disease in man caused by *T. rhodesiense* and that due to *T. gambiense*, except possibly the greater virulence of the former.

3. *T. rhodesiense* is transmitted in Rhodesia by *Glossina morsitans*.

4. Approximately 3·5% of the flies may become permanently infected and capable of transmitting the virus.

5. The period which elapses between the infecting feed of the flies and the date on which they become infective varies from eleven to twenty-five days in the Luangwa Valley.

6. Attempts carried out at laboratory temperature on the Congo-Zambesi plateau during the cold season to transmit the human trypanosome by means of *Glossina morsitans* were invariably unsuccessful in spite of the fact that 680 flies were used in these experiments.

7. The developmental cycle of *T. rhodesiense* in *Glossina morsitans* is to a marked degree influenced by the temperature to which the flies are subjected. High temperatures (75-85° F.) favour the development of the parasite, whilst low temperatures (60°-70° F.) are unfavourable.

8. The first portion of the developmental cycle can proceed at the lower temperatures, but for its completion the higher temperatures are essential.

9. The parasites may persist in the fly at an incomplete stage of their development for at least sixty days under unfavourable climatic conditions.

10. These observations afford an adequate explanation of the extremely long latent periods of trypanosomes in *Glossina* which have occasionally been observed by various workers.

11. The relative humidity of the atmosphere has apparently no influence on the development of the trypanosome in *Glossina morsitans*.

12. Mechanical transmission does not occur if a period of twenty-four hours has elapsed since the infecting meal.

13. *Glossina morsitans*, in nature, has been found to transmit the human trypanosome.

14. The chief reservoir of the human trypanosome is the antelope.

15. The results of examination for the human trypanosome of the blood of a large number of monkeys, wild rats and mice were invariably negative.

DESCRIPTION OF PLATE XVIII

Trypanosoma rhodesiense. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-6. *T. rhodesiense*, 'Human strain,' obtained from a monkey inoculated from a case of Sleeping Sickness.

Figs. 7-12. *T. rhodesiense*, 'Fly strain,' obtained by feeding wild *Glossina morsitans* on healthy animals.

Figs. 13-18. *T. rhodesiense*, 'Game strain,' obtained from game.

SECTION II

TRYPANOSOMES OF GAME AND DOMESTIC STOCK

BY
ALLAN KINGHORN
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Within the confines of the Luangwa Valley a numerous and varied selection of game is found, despite the fact that *Glossina morsitans* is everywhere abundant, but, on the contrary, due doubtless to the presence of these insects, domestic stock is extremely scarce, and in many districts non-existent. A few goats are occasionally found, but some evidence exists to show that these animals are not so insusceptible to trypanosomiasis, under natural conditions, as is locally supposed. Dogs are very seldom seen, and the natives themselves recognise the impossibility of keeping them in the midst of 'fly.' Cattle were seen in one village only.

At Ngoa, on the Congo-Zambesi watershed, game is abundant at certain seasons of the year, and goats and dogs are kept in many of the villages. Although tsetse flies are commonly seen near some of these, such domestic stock animals as exist appear to thrive, though trypanosomes were occasionally found in goats.

(*a*) METHODS

In our experience, trypanosomes are more readily detected in a buck's blood by the examination of thin, stained smears than by that of fresh preparations. Except under unusual circumstances, from one to two hours elapsed after an animal had been shot before its heart reached the laboratory, and this, together with the great heat, had a very deleterious effect on the parasites, destroying their motility, and permitting degenerative changes to occur. In several instances in which fresh preparations, made under these conditions, were examined, as well as blood smears

made in the field and afterwards stained, no trypanosomes were found in the fresh blood, whereas they were present in the permanent preparations.

In making smears, it was found advisable to cut the animal's throat immediately it had been shot, and to obtain the blood from one of the arteries. It is claimed that such films have the following advantages:—

1. Clean, uncontaminated preparations are obtained.

2. The trypanosomes have no opportunity of degenerating, and thus stain more sharply.

3. The preparations are permanent.

4. The parasites can be identified more easily in stained, than in fresh preparations. In this particular the examination of thin films has an obvious advantage over that of thick films.

The preparations were dried in the air, fixed in absolute alcohol, and stained with Giemsa.

Owing to the impossibility of obtaining clean sheep and goats, all the game inoculations were made into monkeys and rats, the amount of blood used varying from 1-10 c.cm. It is recommended that, when possible, sheep and goats be used as well, since they are susceptible to most of the pathogenic trypanosomes, whereas animals such as dogs, monkeys and rats are not. This is an important consideration when dealing with such parasites as *T. vivax* and *T. nanum*.

(*b*) EXAMINATION OF GAME AT NAWALIA AND NGOA

A total of 127 head of game, comprising nineteen genera, was examined at Nawalia, and trypanosomes were found by direct examination, by inoculation, or by both methods, in thirty-three.

At Ngoa, 124 buck, belonging to sixteen genera, were examined, and trypanosomes were found in twenty-one—a percentage of 16·9. Details are given in Tables 22 and 23.

It will be seen that parasites were found at Nawalia by direct examination in twenty-six cases, a percentage of 20·4, while at Ngoa trypanosomes were found in the peripheral blood of only sixteen buck—13·0 %. These are high figures for single observations, and it is probable that had several preparations from each

TABLE 22.—Results of examination of game for trypanosomes at Nawalia.

Animal	Number examined	Number in which trypanosomes were found in buck's blood	Number inoculations made	Number positive inoculations in which parasites were seen in buck's blood	Number positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculations
1. Elephant	1	0	1	0	0	0
2. Rhinoceros	1	0	1	0	0	0
3. Hippopotamus ...	1	0	0	0	0	0
4. Zebra	5	0	3	0	0	0
5. Roan	8	1	2	0	0	1
6. Wildebeest	2	0	1	0	0	0
7. Kudu	7	3	3	1	1	4
8. Hartebeest	6	0	1	0	1	1
9. Waterbuck	28	16	14	5	1	17
10. Puku	10	1	6	0	0	1
11. Mpala...	29	1	13	1	1	2
12. Bushbuck	9	4	6	1	2	6
13. Bushpig	4	0	1	0	0	0
14 Warthog	9	0	3	0	1	1
15. Lion	2	0	0	0	0	0
16. Hunting dog ...	1	0	1	0	0	0
17. Giant rat	1	0	0	0	0	0
18. Genet	2	0	0	0	0	0
19. Squirrel	1	0	0	0	0	0
	127	26	56	8	7	33

Table 23.—Results of examination of game for trypanosomes at Ngoa.

Animal	Number examined	Number in which trypanosomes were found in buck's blood	Number inoculations made	Number positive inoculations in which parasites were seen in buck's blood	Number positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculations
1. Rhinoceros	6	0	3	0	0	0
2. Zebra	17	0	5	0	0	0
3. Buffalo	6	0	3	0	0	0
4. Eland	15	0	12	0	4	4
5. Roan	5	0	3	0	1	1
6. Hartebeest	8	0	4	0	0	0
7. Waterbuck	27	12	15	3	0	12
8. Puku	8	1	6	0	0	1
9. Sitatunga	2	1	0	0	0	1
10. Duiker	9	2	4	0	0	2
11. Klipspringer	2	0	1	0	0	0
12. Warthog	12	0	3	0	0	0
13. Hyaena	2	0	1	0	0	0
14. Caracal	2	0	0	0	0	0
15. Galago	1	0	0	0	0	0
16. Reedbuck	2	0	0	0	0	0
	124	16	60	3	5	21

buck been searched, the percentage of successes would have been much greater. In several instances, only a single trypanosome was found in a film covering the greater part of a slide, and this after a very careful examination extending over two hours.

A more accurate estimate of the percentage of animals harbouring trypanosomes is afforded by considering only those from

which inoculations were made. An analysis of these gives the following figures:—

	Nawalia.	Ngoa.
Number of inoculations made	56 ...	60
Number of positive inoculations in which parasites were found in buck's blood...	8 ...	3
Number of positive inoculations in which no parasites were found in buck's blood	7 ...	5
Number of negative inoculations in which parasites were found in buck's blood ...	6 ...	6
Total number found infected	21 ...	14

These figures show that at least 37·5% (Nawalia) and 23·3% (Ngoa) of the local fauna were infected with trypanosomes. Both *T. vivax* and *T. nanum* have been found in game, and to both these species monkeys and rats are refractory, so that no conclusions can be drawn regarding the presence or absence of these trypanosomes in animals in which parasites were not found in the blood smears. Had sheep and goats been available for inoculation, it is probable that many more buck would have been shown to harbour the two organisms in question. As a conservative estimate, the percentage of game actually infected with trypanosomes in the vicinity of Nawalia might be placed at 50, and at Ngoa 35.

A further point which is brought out in the tables is that different species of buck appear to vary widely in their susceptibility. Amongst the commoner varieties, trypanosomes were never found either by direct examination, or by inoculation in zebra, buffalo, wildebeest and bushpig, and only rarely in roan, hartebeest, puku, mpala and warthog. Waterbuck, eland, bushbuck and kudu were the species found to be most heavily infected.

To a certain extent, perhaps, these differences may be accounted for by the habitats affected by the various species of game. Kudu and bushbuck, and waterbuck to a lesser extent, are usually found in thick cover from which they seldom emerge, and where they are more constantly exposed to the bites of tsetse flies. Mpala, puku and wildebeest are usually found in open country, frequently remaining for the greater part of the day on wide, bare plains, and here the flies are less noticeable than in the bush. Specific

TABLE 24.—Percentages of various species of game found infected with trypanosomes at Nawalia.

Animal	Number examined	Percentage harbouring trypanosomes
Bushbuck	9	66·6
Waterbuck	28	60·7
Kudu	7	57·1
Hartebeest	6	16·6
Roan	8	12·5
Warthog	9	11·1
Puku	10	10·0
Mpala	29	6·9

TABLE 25.—Percentages of various species of game found infected with trypanosomes at Ngoa.

Animal	Number examined	Percentage harbouring trypanosomes
Sitatunga	2	50
Waterbuck	27	44·4
Eland	15	26·6
Duiker	9	22·2
Roan	5	20
Puku	8	12·5

differences in the amount of immunity enjoyed by buck are probably, however, of much greater importance.

In Tables 26 and 27 are given the species of trypanosomes occurring in each animal in which parasites were found. In compiling the tables, information obtained from the result of inoculations, where these were made, has been utilised. This enables a differentiation to be made between such parasites as *T. pecorum* and *T. nanum*, which are morphologically indistinguishable. *T. vivax* has a characteristic morphology, and can thus be identified in blood smears without difficulty.

TABLE 26.—Trypanosomes found in game at Nawalia.

Animal		Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Bushbuck	1	Negative	*T. pecorum*	*T. pecorum*
,,	2	*T. pecorum* or *T. nanum*	Negative	*T. nanum*
,,	3	*T. multiforme*, sp. nov.	*T. multiforme*, sp. nov.	*T. multiforme*, sp. nov.
,,	4	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,,	5	*T. pecorum* or *T. nanum*	,,	*T. pecorum* or *T. nanum*
,,	6	Negative	*T. rhodesiense*	*T. rhodesiense*
Waterbuck	1	*T. pecorum* or *T. nanum*	*T. pecorum*	*T. pecorum*
,,	2	*T. pecorum* or *T. nanum*	*T. pecorum* and *T. rhodesiense*	*T. pecorum* and *T. rhodesiense*
,,	3	*T. pecorum* or *T. nanum* and *T. vivax*	Negative	*T. nanum* and *T. vivax*
,,	4	*T. pecorum* or *T. nanum*	,,	*T. nanum*
,,	5	Negative	*T. pecorum*	*T. pecorum*
,,	6	*T. vivax*	Negative	*T. vivax*
,,	7	*T. vivax*	,,	*T. vivax*
,,	8	*T. rhodesiense*	*T. rhodesiense*	*T. rhodesiense*
,,	9	*T. pecorum* or *T. nanum* and *T. vivax*	Negative	*T. nanum* and *T. vivax*
,,	10	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,,	11	(?) *T. rhodesiense*	Animal died day after inoculation	(?) *T. rhodesiense*
,,	12	*T. rhodesiense*	*T. rhodesiense* and *T. pecorum*	*T. rhodesiense* and *T. pecorum*
,,	13	*T. vivax*	No inoculation	*T. vivax*
,,	14	*T. rhodesiense* and *T. vivax*	*T. rhodesiense*	*T. rhodesiense* and *T. vivax*
,,	15	*T. vivax*	No inoculation	*T. vivax*
,,	16	(?) *T. rhodesiense* and *T. vivax*	,,	(?) *T. rhodesiense* and *T. vivax*
,,	17	(?) *T. rhodesiense*	,,	(?) *T. rhodesiense*
Kudu	1	Negative	*T. pecorum*	*T. pecorum*
,,	2	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,,	3	*T. pecorum* or *T. nanum*	*T. pecorum*	*T. pecorum*
,,	4	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
Roan	1	*T. pecorum* or *T. nanum*	,,	*T. pecorum* or *T. nanum*
Warthog	1	Negative	*T. rhodesiense*	*T. rhodesiense*
Puku	1	*T. vivax*	No inoculation	*T. vivax*
Mpala	1	Negative	*T. rhodesiense*	*T. rhodesiense*
,,	2	*T. pecorum* or *T. nanum*	*T. pecorum* or *T. rhodesiense*	*T. pecorum* and *T. rhodesiense*
Hartebeest	1	Negative	*T. rhodesiense*	*T. rhodesiense*

TABLE 27.—Trypanosomes found in game at Ngoa.

Animal		Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculations into monkeys and rats	Diagnosis
Waterbuck	1	*T. vivax*	No inoculation	*T. vivax*
,,	2	*T. vivax*	,,	*T. vivax*
,,	3	*T. vivax*	,,	*T. vivax*
,,	4	*T. vivax*	Negative	*T. vivax*
,,	5	*T. vivax*	,,	*T. vivax*
,,	6	*T. vivax*	*T. rhodesiense*	*T. vivax* and *T. rhodesiense*
,,	7	*T vivax*	*T. rhodesiense*	*T. vivax* and *T. rhodesiense*
,,	8	*T. vivax*	Negative	*T. vivax*
,,	9	*T. vivax*	*T. pecorum*	*T. vivax* and *T. pecorum*
,,	10	*T. vivax*	Negative	*T. vivax*
,,	11	*T. vivax*	No inoculation	*T. vivax*
,,	12	*T. vivax*	,,	*T. vivax*
Eland	1	Negative	*T. pecorum*	*T. pecorum*
,,	2	,,	*T. pecorum*	*T. pecorum*
,,	3	,,	*T. pecorum*	*T. pecorum*
,,	4	,,	*T. pecorum*	*T. pecorum*
Roan	1	,,	*T. pecorum*	*T. pecorum*
Puku	1	*T. vivax*	Negative	*T. vivax*
Sitatunga	1	*T. tragelaphi*, sp. nov.	No inoculation	*T. tragelaphi*, sp. nov.
Duiker	1	*T. vivax*	Negative	*T. vivax*
,,	2	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*

As would be expected, double infections in game are not uncommon, and several instances of this are recorded in the tables.

No data exist as to the ultimate effect of infection on game. All the animals which were shot appeared to be in perfect condition, and presented no objective signs of disease. Whether

or not buck succumb to trypanosomiasis it is impossible to say, but as they have increased steadily since rinderpest swept through the country, it may be assumed that their tolerance to trypanosomes is very great.

(c) EXAMINATION OF DOMESTIC STOCK

The domestic animals examined, and the species of trypanosomes found in them, are given in Tables 28 and 29.

TABLE 28.—Examination of domestic stock for trypanosomes at Nawalia.

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Cow	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,, ...	*T. pecorum* or *T. nanum*	,,	*T. pecorum* or *T. nanum*
Goat ... 39	*T. vivax*	Negative	*T. vivax*
,, ... 94	*T. vivax* and *T. nanum* or *T. pecorum*	,,	*T. vivax* and *T. nanum*
,, ... 202	*T. pecorum* or *T. nanum*	Negative	*T. nanum*
,, ... 258	*T. vivax*	,,	*T. vivax*
Dog	*T. rhodesiense*	*T. rhodesiense*	*T. rhodesiense*
,, ...	*T. pecorum*	*T. pecorum*	*T. pecorum*
,, ...	*T. pecorum*	No inoculation	*T. pecorum*
,, ...	*T. pecorum*	,,	*T. pecorum*
,, ...	*T.* sp. (*montgomeryi* ?)	Negative	*T.* sp. (*montgomeryi* ?)

TABLE 29.—Examination of domestic stock for trypanosomes at Ngoa.

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Goat ... 369	*T. nanum* or *T. pecorum*	No inoculation	*T. nanum* or *T. pecorum*
,, ... 375	*T. vivax* and *T. nanum* or *T. pecorum*	,,	*T. vivax* and *T. nanum* or *T. pecorum*
,, 378	*T. vivax* and *T. nanum* or *T. pecorum*	*T. pecorum*	*T. vivax* and *T. pecorum*

The only native village in which cattle were found was Kambwiri's, some forty miles south-west of Nawalia. At present there are only three head, all that are left of a big herd which existed there some four or five years ago. Two of the three appeared to be in good condition when seen, but the headman of the village fully expected to lose them within a few months. The third beast was obviously ill. The cow in which trypanosomes were found at Fort Jameson was bred on the Government Farm, and had never been beyond the limits of the township. Tsetse have never been seen within some miles of the place, but *Stomoxys* is abundant in the kraals, and at certain seasons of the year various species of *Tabanidae* are common.

In several of the villages on the main road from Nawalia to Fort Jameson, a number of goats were found at the end of August, 1911, and again at the beginning of April, 1912, but at the end of that month not a single animal was alive. *Glossina morsitans* was found around all these villages. The four goats mentioned in Table 28 were under observation at Nawalia for a considerable length of time. During this period, parasites were found in the peripheral blood only at rare intervals. Two were rather thin, but not markedly so, and, apart from this, there were no signs of disease. Goat No. 258 was examined at frequent intervals for two months before parasites were first found, while in the others, trypanosomes were seen on the first occasion. Nos. 39 and 258, after having been under observation for nine and four months respectively, died on the road when the Commission left Nawalia, most probably from being over-driven. The other two are still alive, seven and four months after the diagnosis was made.

The dog in which *T. rhodesiense* was found came from a village just on the Nyasaland border. The natives said that it had not been out of the village for over a year previously. As the disease runs an extremely acute course in these animals, there can be no doubt that the dog was infected locally.

(*d*) EXAMINATION OF SMALL VERMIN

It has been suggested that the small vermin might also act as reservoirs of trypanosomiasis. It must be remembered, however, that many of the small vermin of Tropical Africa are nocturnal, and are, therefore, not subjected to the same extent as are the big game to the bites of *Gl. morsitans*. At Nawalia and at Ngoa we examined in all 142 wild rats, 15 wild mice, 1 wild rabbit, 1 giant rat, 1 squirrel, 1 galago, and 2 genet; the results were uniformly negative. Furthermore, it might be remarked that there is no evidence to show that the small vermin are tolerant of the human trypanosome as are the big game. In those which we infected experimentally the disease ran an acute course, and the animals died. If this be the case with the majority of the small vermin they cannot have the same significance as reservoirs of the virus as have the big game, which can probably harbour the parasite for long periods without exhibiting signs of disease.

Not a single case of infection with trypanosomes was found in the 256 monkeys (*Cercopithecus pygerythus*) examined by us, although infection with filaria and *Plasmodium kochi* was common.

The probable explanation of this is that the monkeys during the daytime catch the tsetse fly before the insects have time to feed on them, whereas, on the other hand, they are frequently bitten by mosquitos whilst they are asleep at night. Moreover, it must be remembered that in these animals infection with the human trypanosome runs an acute course, and those animals which contract the disease quickly succumb.

SUMMARY

Trypanosomes are of frequent occurrence in game and domestic stock in North Eastern Rhodesia. As a conservative estimate the percentage of big game infected with trypanosomes pathogenic to man and domestic stock may at Nawalia (Luangwa Valley) be placed at 50, and at Ngoa (Congo-Zambesi watershed) at 35.

At Nawalia six species of trypanosomes were isolated from game

and domestic stock, viz., *T. rhodesiense*, *T. vivax*, *T. nanum*, *T. pecorum*, *T. montgomeryi*, and *T. multiforme;* whilst at Ngoa five species were found, viz., *T. rhodesiense*, *T. vivax*, *T. nanum*, *T. pecorum*, and *T. tragelaphi*.

The results of examination of over 400 monkeys, wild rats and mice were invariably negative.

SECTION III

TRYPANOSOMES FOUND IN WILD *GLOSSINA MORSITANS*

BY

ALLAN KINGHORN

AND

WARRINGTON YORKE

During the sojourn of the Commission at Nawalia and at Ngoa, experiments were undertaken with the object of ascertaining the species of trypanosomes transmitted, in nature, by *Glossina morsitans*, Westw. The flies, as they were brought to the laboratory, were fed on clean monkeys, which were the only animals available for the purpose. Unfortunately, owing to the lack of healthy goats and sheep, no definite conclusions can be drawn as to whether the fly was infected with such species as *Trypanosoma vivax* and *Trypanosoma nanum*, both of which are of common occurrence in game and domestic stock.

At Nawalia, in the Luangwa Valley, freshly-caught *Glossina morsitans* were fed on healthy monkeys from day to day. In all, 3,410 flies were fed in batches, as they were brought to the laboratory, on thirty-three monkeys, but as five of the latter died within two or three days, inferences can only be drawn as to the infectivity of the 3,202 flies fed on the remaining twenty-nine animals. Details of the experiments are given in Table 30.

It will be seen from the table that three species of trypanosomes were isolated, namely, *Trypanosoma rhodesiense*, *Trypanosoma pecorum*, and a third, hitherto undescribed parasite, for which we propose the name *Trypanosoma ignotum*.

TABLE 30.—Result of feeding freshly-caught *Glossina morsitans* on healthy monkeys at Nawalia.

Number of Experiment	Date	Number of flies fed	Result	Trypanosomes isolated
1	21.6.11	18	Negative	
4	5.7.11	128	Infection	*T. ignotum, sp. nov.*
7	19.7.11	97	Negative	
28	28.7.11	160	Infection	*T. ignotum, sp. nov.*
35	7.8.11	193	,,	*T. ignotum. sp. nov.*
95	30.10.11	90	Negative	
96	30.10.11	82	Infection	*T. rhodesiense*
121	14.11.11	98	Negative	
143	4.12.11	100	,,	
100	6.12.11	105	Infection	? *T. rhodesiense*, animal died day after becoming infected
215	13.1.12	41	,,	*T. ignotum, sp. nov.*
210	7-12.1.12	269	,,	*T. rhodesiense*
217	16.1.12	200	,,	*T. rhodesiense*
224	23.1.12	28	Negative	
259	13.2.12	104	,,	
316	20.3.12	101	Infection	*T. rhodesiense*
317	21.3.12	112	,,	*T. pecorum*
326	25.3.12	93	Negative	
329	26.3.12	130	Infection	*T. ignotum, sp. nov.*
330	27.3.12	137	,,	*T. ignotum, sp. nov.*
333	28.3.12	74	,,	*T. rhodesiense*
334	29.3.12	67	Negative	
336	30.3.12	109	Infection	*T. ignotum, sp. nov.*
340	1.4.12	108	,,	*T. ignotum, sp. nov.*
342	2.4.12	52	Negative	
343	3.4.12	85	Infection	*T. rhodesiense* and *T. ignotum, sp. nov.*
348	4.4.12	90	Negative	
353	6.4.12	137	Infection	*T. pecorum* and *T. ignotum, sp. nov.*
94	15.9.11	194	—	Goat; subinoculated monkeys and rats did not become infected

An analysis shows that of the 3,202 flies used, at least nineteen were capable of infecting monkeys. This figure is based on the assumption that, with the exception of Experiments Nos. 343 and 353, each batch contained but a single infective fly. In each of these experiments it is highly probable that at least two infective flies were present, as in the former both *Trypanosoma rhodesiense* and *Trypanosoma ignotum*, sp. nov., were found in the monkey's blood, and in the latter both *Trypanosoma pecorum* and *Trypanosoma ignotum*, sp. nov. The percentage of flies infected with each of the three trypanosomes is given in tabular form.

TABLE 31.—Proportion of wild *Glossina morsitans* infected with *T. rhodesiense*, *T. pecorum* and *T. ignotum*, *sp. nov.*

Species	Number of flies fed	Number of infections	Ratio of infected to non-infected flies
T. ignotum	3,008	10	1 : 300
T. rhodesiense	3,202	6*	1 : 534
T. pecorum	3,202	2	1 : 1600

In our second interim report* one experiment in which *T. rhodesiense* was obtained was inadvertently omitted, so that this parasite was isolated in six, instead of five instances. In addition, Monkey No. 100 became infected four days after the flies had been fed on it. As the animal died the same day, we were unable to decide the species of trypanosome present, but from the short inoculation it is highly probable that the parasite was *T. rhodesiense*. On this assumption the ratio of infected to non-infected flies would be 1 to 455.

At Ngoa, on the Congo-Zambesi watershed, 5,250 freshly-caught *Glossina morsitans* were fed in batches on forty monkeys and two goats. Details are given in Table 32.

* Kinghorn, A., and Yorke, W. A Further Report on the Transmission of Human Trypanosomes by *Glossina morsitans*, Annals of Tropical Medicine and Parasitology, 1912, Vol. VI, p. 269.

TABLE 32.—Results of feeding freshly-caught *Glossina morsitans* on healthy monkeys at Ngoa.

Number of Experiment	Date	Number of flies fed	Result	Trypanosomes isolated
2 goats	May	248	Negative	Subinoculated monkeys did not become infected with *T. rhodesiense* or *T. pecorum*
366	23.4.12	223	Infection	*T. ignotum*
367	23.4.12	212	,,	*T. ignotum*
368	23.4.12	211	,,	*T. ignotum*
377	4.5.12	130	Negative	
379	6.5.12	121	Infection	*T. ignotum*
395	16.5.12	124	Negative	*T. ignotum*
404	28.5.12	146	Infection	*T. rhodesiense* and *T. ignotum*
405	29.5.12	109	,,	*T. ignotum*
412	1.6.12	90	Negative	
421	4.6.12	151	Infection	*T. ignotum*
423	6.6.12	166	,,	*T. ignotum*
429	11.6.12	92	Negative	
430	12.6.12	120	,,	
436	14.6.12	145	,,	
439	17.6.12	136	,,	
444	19.6.12	159	,,	
446	21.6.12	85	,,	
456	24.6.12	120	,,	
460	25.6.12	122	,,	
461	26.6.12	116	Infection	*T. rhodesiense*
464	28.6.12	110	Negative	
465	28.6.12	78	,,	
466	29.6.12	84	,,	
469	1.7.12	118	Infection	*T. ignotum*
471	2.7.12	64	Negative	
476	4.7.12	70	,,	

TABLE 32.—*continued.*

Number of Experiment	Date	Number of flies fed	Result	Trypanosomes isolated
481	5.7.12	80	Negative	
485	6.7.12	96	,,	
487	8.7.12	162	Infection	*T. rhodesiense*
502	11.7.12	104	,,	*T. ignotum* and *T. pecorum*
503	12.7.12	168	Negative	
506	13.7.12	121	,,	
511	15.7.12	84	Infection	*T. ignotum*
514	16.7.12	123	Negative	
515	17.7.12	140	Infection	*T. ignotum*
518	19.7.12	135	,,	*T. ignotum*
521	19.7.12	118	,,	*T. ignotum*
524	20.7.12	160	,,	*T. rhodesiense* and *T. ignotum*
559	2.8.12	118	Negative	
560	3.8.12	93	,,	

It will be seen from Table 33 that *T. rhodesiense*, *T. pecorum* and *T. ignotum* were isolated from the plateau flies in the proportion of 1 in 312, 1 in 1312, and 1 in 5250 respectively.

TABLE 33.—Proportion of wild *Glossina morsitans* infected with *T. ignotum*, *T. rhodesiense* and *T. pecorum*.

Species	Number of flies fed	Number of infections	Ratio of infected to non-infected flies
T. ignotum	5,000	16	1 : 312
T. rhodesiense	5,250	4	1 : 1312
T. pecorum	5,250	1	1 : 5250

SUMMARY

T. rhodesiense, *T. ignotum* and *T. pecorum* are transmitted by *Glossina morsitans* in nature, and were obtained by feeding wild freshly-caught *Glossina morsitans* on healthy monkeys.

SECTION IV

DESCRIPTION OF TRYPANOSOMES FOUND

BY
ALLAN KINGHORN
AND
WARRINGTON YORKE

1. *TRYPANOSOMA RHODESIENSE* (Pl. XVIII)

This parasite has been fully dealt with in a previous section, and requires, therefore, no further description. It was isolated from all the cases of Sleeping Sickness, sixteen in number, observed by the Commission. At Nawalia it was found in four waterbuck, two mpala, one hartebeest, one bushbuck and one warthog—16 % of the game from which inoculations were made—and from one native dog. Parasites resembling *T. rhodesiense* were found in blood films made from three other waterbuck, from which no sub-inoculations were made. At least six, and possibly seven, of 3,202 freshly-caught *Glossina morsitans* were found capable of transmitting this organism. At Ngoa the trypanosome was isolated from two waterbuck, and from four of 5,250 freshly-caught tsetse flies.

2. *TRYPANOSOMA VIVAX* (Pl. XIX, figs. 1-8)

At Nawalia this organism was found in eight waterbuck, one puku and three goats, and at Ngoa in twelve waterbuck, one puku, one duiker and two goats.

MORPHOLOGY

(*a*) In fresh preparations it appears as a club-shaped organism, characterised chiefly by the extraordinary rapidity with which it moves across the field.

(*b*) In stained preparations it is seen to be more or less club-shaped, with a long free flagellum. The greatest width is posterior to the nucleus, which is situated about the middle of the body.

TABLE 34.—Measurements of *T. vivax*.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Goat ... 39	?	25	24·35	26	22·5
.. ... 39	?	25	23·67	26·25	21·25
,, ... 258	?	25	22·43	25·5	19·75
,, ... 258	?	25	22·74	25·5	21·25
,, ... 258	?	25	23·48	28·25	20·25
,, ... 448	17	25	24·69	28·25	18·75
,, ... 448	18	25	24·5	28·75	20·25
,, ... 448	19	25	23·22	26·5	19·25
		200	23·63	28·75	18·75

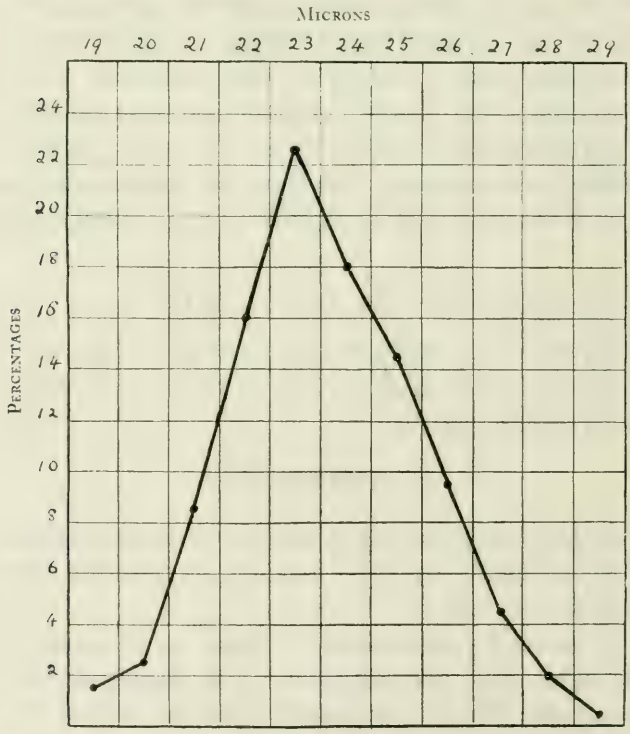


CHART 2.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma vivax*.

The blepharoplast is large and rounded, and lies close to the posterior extremity of the parasite. The undulating membrane is, as a rule, very feebly developed, or absent.

The mean length of 200 trypanosomes was 23·6μ, the maximum 28·75μ, and the minimum 18·75μ (see Table 34). The greatest width varied between 2 and 4·25μ, average 3·2μ.

PATHOGENICITY

Inoculations were made into the following animals:—

8 monkeys	...	...	...	all remained negative.
2 rabbits	...	...	...	both ,, ,,
8 rats ...	...	...	...	all ,, ,,

TRANSMISSION

Owing to the fact that we were unable to obtain a number of clean goats, we could not ascertain definitely that *Glossina morsitans* transmitted this trypanosome in nature. Nevertheless, that this fly can transmit *T. vivax* is shown by the following two experiments. The wild *Glossina morsitans* used for breeding purposes were fed regularly for over two months on goats which were naturally infected with *T. vivax* and *T. pecorum*. These goats were obtained from Ngoa, in the vicinity of which tsetse flies were abundant.

Experiment 1

On July 3rd, forty-eight of the breeding flies, which had previously been starved for five days, were fed on a young, healthy goat. The goat's blood had been examined regularly for ten days before the commencement of the experiment, and no parasites were found. Ten days later the animal became infected with *Trypanosoma vivax*.

Experiment 2

Three other flies were fed on a healthy kid on June 24th, and twelve days later *T. vivax* appeared in the peripheral circulation. The insects were dissected on the day after they had fed on the goat, and trypanosomes were found in one only. The infection

was confined to the proboscis, in which the parasites were extremely numerous and disposed in large rosettes.

Both these goats were brought, in mosquito-proof cages, from a fly-free area.

3. *TRYPANOSOMA NANUM* (Pl. XIX, figs. 9-16)

Found in the following animals at Nawalia:—One bushbuck, three waterbuck and two goats. Possibly it was also present in two other bushbuck, one other waterbuck, two kudu, a roan and two cattle, but as no sub-inoculations were made, it was impossible to differentiate it from *Trypanosoma pecorum*. At Ngoa the parasite may have been present in a duiker and two goats, but in the absence of sub-inoculations it could not be distinguished from *T. pecorum*.

MORPHOLOGY

(*a*) In fresh preparations it appears as a short, sluggish organism. As a rule, it does not progress.

(*b*) In stained preparations it is found to be short, with a more or less rounded posterior extremity from which the body tapers forwards to the acute anterior end; the nucleus is placed at the centre of the body. The blepharoplast is small, and is situated near the posterior extremity. There is no free flagellum, and the undulating membrane is absent, or, at most, very slightly developed. The protoplasm is free of vacuoles and granules, in general.

The mean length of 200 individuals was 14·3μ, the maximum 19μ, and the minimum 10μ (see Table 35). The breadth, at the level of the nucleus, varied from 1 to 2·25μ, the average being 1·5μ.

PATHOGENICITY

The following animals were inoculated:—

3 monkeys	...	...	...	all remained negative.		
1 rabbit	...	...	...	,,	,,	,,
3 rats	...	...	...	,,	,,	,,

TABLE 35.—Measurements of *T. nanum*.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Goat ... 202	Naturally infected	25	15·2	19·0	12·25
,, ... 202	,,	25	14·77	18·5	11·5
,, ... 202	,,	25	14·0	17·0	11·5
,, ... 202	,,	25	14·61	18·0	11·5
,, ... 202	,,	25	13·99	17·0	11·5
,, ... 202	,,	25	13·51	18·0	10·5
,, ... 202	,,	25	14·43	18·75	10·0
,, ... 202	,,	25	14·39	17·0	12·0
		200	14·36	19·0	10·0

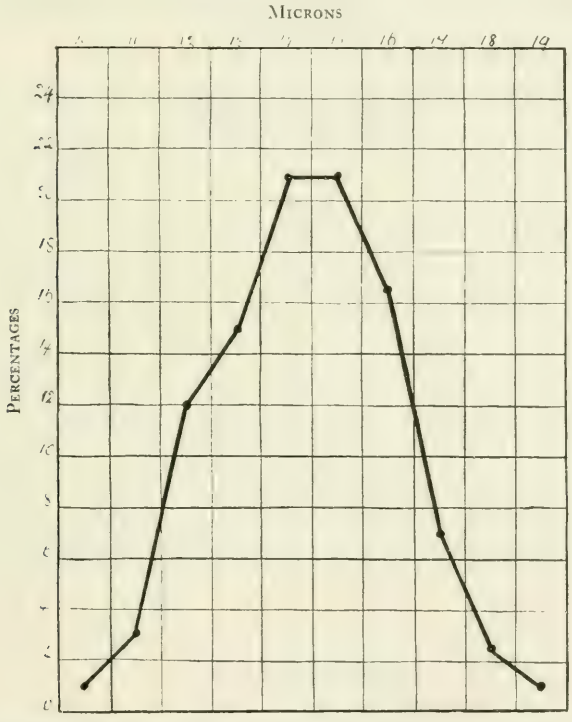


CHART 3.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma nanum*.

DESCRIPTION OF PLATE XIX

Trypanosoma vivax and *Trypanosoma nanum*. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-8. *T. vivax*.

Figs. 9-16. *T. nanum*.

TRANSMISSION

The vector of *T. nanum* has not been determined with certainty.

4. *TRYPANOSOMA PECORUM* (Pl. XX, figs. 1-8)

At Nawalia this parasite was found in one bushbuck, one mpala, four waterbuck, two kudu, three dogs and one wild rat. It was possibly present in two other bushbuck, a fifth waterbuck, two other kudu, a roan and two cattle, but as mentioned before, it could not be distinguished from *T. nanum* in the absence of sub-inoculations. It was also isolated from 2 of 3,202 freshly-caught *Glossina morsitans*.

At Ngoa it was obtained from one waterbuck, four eland, one roan and one goat. It was possibly present in one duiker and two other goats. It was also found in one wild tsetse fly.

MORPHOLOGY

(*a*) In fresh preparations, this parasite resembles *T. nanum* very closely. It is a short, sluggish organism, showing no degree of translatory power.

(*b*) In stained preparations it appears as a short organism, with an obtuse or rounded posterior extremity from which it tapers to the attenuated anterior end. The nucleus is oval or rounded, and situated near the middle of the body. The blepharoplast is small and rounded, and lies close to the posterior extremity. The undulating membrane, if present, is very feebly marked, and there is no free flagellum. The general protoplasm stains uniformly, and is devoid of granules and vacuoles.

The mean length of 500 individuals was 13·6μ, the maximum 19μ, and the minimum 9·5μ (see Table 36). The breadth, at the level of the nucleus, varied from 1 to 2·5μ, the average being 1·5μ.

PATHOGENICITY

A synopsis of the pathogenicity is given in Table 37, p. 253.

TABLE 36.—Measurements of *T. pecorum*.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Ox	393	11	25	13·73	18·25	10·25
,,	393	14	25	12·92	15·5	9·75
Monkey	2	21	25	13·27	16·25	10·0
,,	9	9	25	13·0	16·8	10·3
,,	37	29	25	14·47	16·75	11·0
,,	109	27	25	13·37	16·75	10·5
,,	109	36	25	13·09	15·75	10·75
,,	123	28	25	15·26	19·0	12·0
,,	317	43	25	14·28	17·5	11·0
,,	317	47	25	13·54	17·5	11·0
,,	339	14	25	15·61	17·75	11·75
Dog	274	(?) Naturally infected	25	14·09	16·75	11·0
Guinea-pig	31	21	25	12·33	15·5	9·5
Rat	49	9	25	13·17	16·0	9·8
,,	163	20	25	13·26	15·25	10·75
,,	163	23	25	13·13	16·25	10·75
,,	282	5	25	12·38	14·75	9·5
,,	282	11	25	13·75	16·0	10·25
Mouse	11	12	25	13·4	16·0	10·6
,,	12	5	25	14·1	16·8	9·8
			500	13·6	19·0	9·5

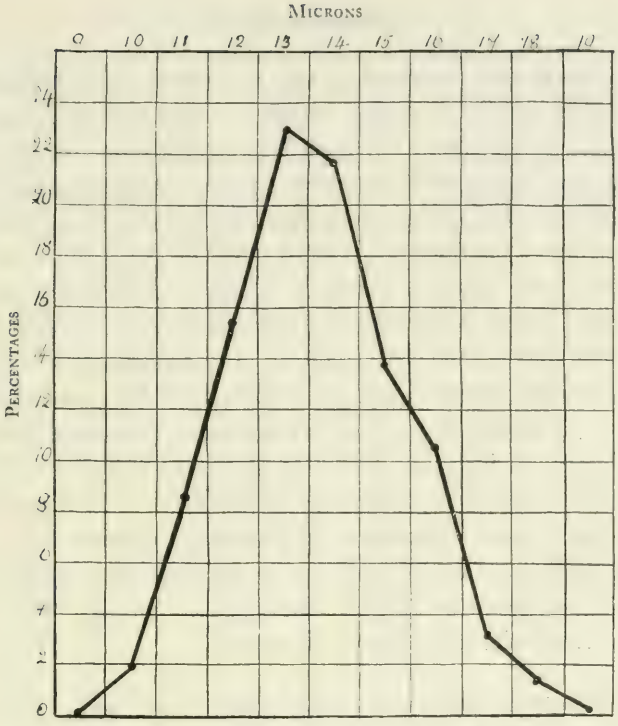


CHART 4.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma pecorum*.

TABLE 37.—Pathogenicity of *Trypanosoma pecorum*.

Animal	No. inoculated	Incubation period days	Duration days
Monkey	17	8-21, average 12½	23-225 (still alive)
Rabbit	3	8-11, ,, 10	66-215
Guinea-pig	1	5	39
Rat	14	3-14, average 6½	6-32
Mouse	2	5	27-75
Goat	3	11-18, average 15	53-58 (still alive)
Ox	1	9	

TRANSMISSION

T. pecorum was obtained in two of twenty-eight experiments in which freshly-caught *Glossina morsitans* were fed on clean monkeys at Nawalia, and in one of forty experiments at Ngoa. In all, therefore, 3 of 8,452 wild flies were found to be naturally infected.

At Ngoa batches of breeding flies which had fed for two to three months on goats, naturally-infected with *T. pecorum*, were allowed to feed on three healthy goats. That on which the first batch, consisting of thirty flies, were fed became infected with the parasite on the 11th day of the experiment. Trypanosomes were found in the blood of the second goat, on which eight flies were fed, on the 12th day. The third goat, on which three flies were fed, became infected after an incubation period of eighteen days. The flies in the last two groups were dissected the day after they had fed. Trypanosomes were found in the gut and proboscis of a number, but in no instance was an infection of the salivary glands observed.

These experiments afford satisfactory proof that *Glossina morsitans* transmits the parasite in nature.

5. *TRYPANOSOMA MULTIFORME*, sp. nov.
(Pl. XX, figs. 9-16)

This parasite was isolated from a bushbuck at Nawalia.

MORPHOLOGY

(*a*) In fresh preparations, the trypanosome is seen to be markedly polymorphic, resembling in this particular *Trypanosoma rhodesiense*, *Trypanosoma gambiense* and *Trypanosoma pecaudi*. Short sluggishly-moving forms are seen, as well as long free-flagellated, active ones. As a rule, the short varieties are more or less stationary, while the long ones progress fairly rapidly. The proportions of each of the forms varies widely, from day to day, in any one animal.

(*b*) In stained preparations, every gradation between extremely short, aflagellar forms, indistinguishable from *T. pecorum*, and long, slender, free-flagellated parasites are seen. In general, the

trypanosome morphologically closely resembles *T. gambiense* or *T. brucei*, except for the presence of occasional *pecorum*-like forms.

The mean length of 1,000 individuals was 21·18μ, the maximum 33·5μ, and the minimum 10·5μ. The curve (Chart 5, p. 259) representing the distribution of the various lengths of the trypanosomes, expressed in percentages of the total number measured (1,000) differs from that of *T. gambiense* in that the apex occurs at 17μ.

TABLE 38.—Measurements of *T. multiforme*, sp. nov.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Monkey 166	47	25	18·26	26·0	14·25
,, 166	62	25	16·2	18·5	13·75
,, 166	78	25	15·34	18·0	12·0
,, 312	12	25	17·93	24·75	10·75
,, 312	13	25	16·84	23·0	13·75
,, 312	19	25	18·09	27·5	15·75
,, 312	42	25	19·65	28·0	17·25
,, 312	23	25	24·73	30·25	13·0
,, 312	44	25	17·48	28·0	13·0
,, 360	9	25	23·73	30·75	15·0
,, 360	14	25	18·88	24·5	14·75
,, 360	19	25	20·22	27·75	15·5
Rabbit 370	7	25	18·36	28·25	11·75
,, 370	15	25	24·1	29·75	16·25
,, 370	16	25	20·55	29·0	13·25
,, 370	81	25	23·49	28·75	19·5
,, 414	5	25	25·82	33·5	14·0
,, 414	12	25	25·76	31·0	15·5
,, 414	13	25	23·42	30·0	12·0
,, 414	16	25	21·1	29·25	10·5

TABLE 38.—*T. multiforme*, continued.

Animal	Day of disease	Number measured	Length in Microns		
			Average	Maximum	Minimum
Rabbit 414	18	25	21·49	28·5	13·75
,, 414	22	25	26·03	32·25	18·25
,, 414	71	25	26·41	31·5	20·0
,, 414	78	25	25·84	30·5	17·25
,, 414	79	25	23·8	29·5	12·0
,, 414	81	25	21·44	28·75	15·0
Rat 219	7	25	21·75	31·25	23·75
,, 219	13	25	24·79	32·0	16·25
,, 219	32	25	20·32	26·25	15·75
,, 219	42	25	18·54	26·0	14·0
,, 219	43	25	20·0	30·0	14·25
,, 219	44	25	16·61	23·25	12·75
,, 219	45	25	16·08	25·75	12·75
,, 253	12	25	25·38	33·0	15·75
,, 253	13	25	22·11	33·25	15·75
,, 253	19	25	21·46	30·25	13·25
,, 361	11	25	20·97	32·25	17·25
,, 361	21	25	21·64	30·0	16·0
,, 361	40	25	19·23	30·0	15·25
,, 361	53	25	17·24	19·5	12·5
		1,000	21·18	33·5	10·5

TABLE 39.—Pathogenicity of *Trypanosoma multiforme*.

Animal		Incubation period days	Duration days	Remarks
Monkey	166	8	94	Tryps. last seen on 78th day.
,,	312	10	74	Tryps. last seen on 55th day.
,,	360	7	156	Tryps. last seen on 65th day.
,,	455	4	Still alive 110th day	Tryps. last seen on 16th day.
Rabbit	370	6	119	Tryps. last seen on 112th day.
,,	414	—	—	Did not become infected.
		5 after 2nd inoculation	69	Tryps. present at time of death.
Guinea-pig	371	—	—	Did not become infected. Inoculated twice.
,,	413	—	—	,, ,,
,,	454	—	—	,, ,,
Rat	219	7	104	Tryps. last seen 44th day.
,,	253	12	125	Tryps. last seen 86th day.
,,	361	10	67	Tryps. last seen 61st day.
Ox	391	—	—	Did not become infected. Inoculated twice.
Goat	501	—	—	,, ,,

DIAGNOSIS

This parasite is at once distinguished from *T. pecaudi* and *T. rhodesiense* by the absence of posterior nuclear forms and by its slight pathogenicity for laboratory animals. It more closely resembles *T. gambiense* than any other known species, and is not easily distinguished from this parasite. A study of the biometric curves of the two trypanosomes shows certain differences, and if the percentages of short, intermediate and long forms of each be compared, as in Table 40, the difference is more clearly brought out, the parasite in question exhibiting very few intermediate forms.

TABLE 40.—Comparison of percentages of 'short and stumpy,' 'intermediate,' and 'long' forms of *T. multiforme* and *T. gambiense*.

	Short and stumpy 10—21μ	Intermediate 22—24μ	Long and slender 25—33μ
Trypanosoma multiforme ...	55·6	12·5	31·9
Trypanosoma gambiense ...	51·2	23·1	25·7

The extreme chronicity of the disease caused by this parasite in laboratory animals is an additional point of distinction from *T. gambiense*. We conclude, therefore, that the parasite is a new species, and in view of its marked polymorphism we propose for it the name *Trypanosoma multiforme*.

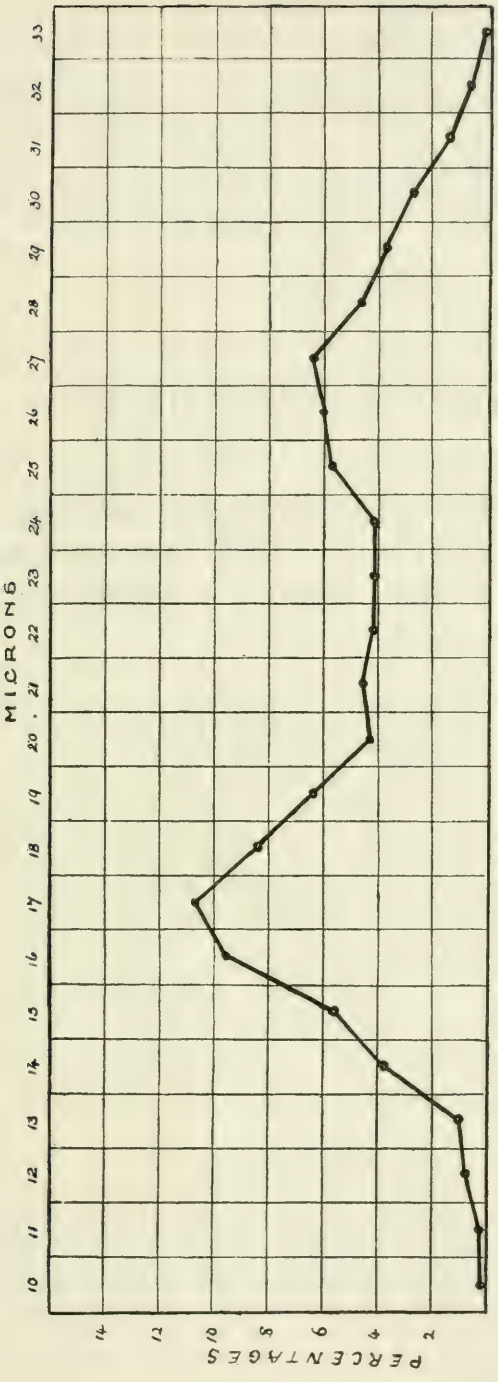


Chart 5.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma multiforme*.

DESCRIPTION OF PLATE XX

Trypanosoma pecorum and *Trypanosoma multiforme*. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-8. *T. pecorum.*

Figs. 9-16. *T. multiforme.*

TRANSMISSION

The vector of *T. multiforme* is unknown.

6. *TRYPANOSOMA SP.* (? *MONTGOMERYI*)
(Pl. XXI, figs.9-16)

Found in one dog, which was obtained from a village in the hills on the Nyasaland border.

MORPHOLOGY

(*a*) In fresh preparations, the parasite appears as a broad, stumpy organism, which shows no marked degree of translatory power.

(*b*) In stained preparations, the trypanosome resembles at first sight *T. pecorum*, but on closer examination it can be readily distinguished from this parasite by its great breadth. The ratio of the breadth to the length is 1 : 4·8. The posterior extremity is subacute or rounded, the anterior attenuated. The greatest width lies at the level of the nucleus, which is situated at the middle of the body, or posterior to it. The blepharoplast is very large and rounded, and is situated near the posterior extremity. Frequently it lies at one edge of the trypanosome, and projects laterally as a small excrescence. The undulating membrane is, as a rule, absent, and when present is simple, and feebly developed. Occasionally a short free flagellum, 1-2μ in length, is to be seen, but this is generally absent. The cytoplasm often contains coarse granules and vacuoles. The latter are most commonly seen in the posterior portion of the body, whereas the granules may be scattered generally throughout the protoplasm.

The average length of 200 individuals was 15·88μ, the maximum 20μ, and the minimum 10μ. The breadth, at the level of the nucleus, varied from 1·25-6·5μ, the average being 3·29μ.

PATHOGENICITY

One rat was sub-inoculated from the dog, and had not become infected up to the 13th day afterwards, when it was accidentally killed. Unfortunately the dog died some days previously, so that the strain was lost.

TABLE 41.—Measurements of *T. montgomeryi*.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Dog 206	Naturally-infected 27.1.12	25	15·56	18·25	12·5
.. 206	,, 28.1.12	25	15·83	20·0	12·5
.. 206	,, 29.1.12	25	15·53	18·5	11·75
.. 206	,, 30.1.12	25	16·88	19·25	14·0
.. 206	,, 31.1.12	25	16·84	19·0	14·0
.. 206	,, 1.2.12	25	14·72	17·75	11·5
.. 206	,, 3.2.12	25	15·20	17·75	10·0
.. 206	,, 4.2.12	25	16·55	18·75	13·5
		200	15·88	20·0	10·0

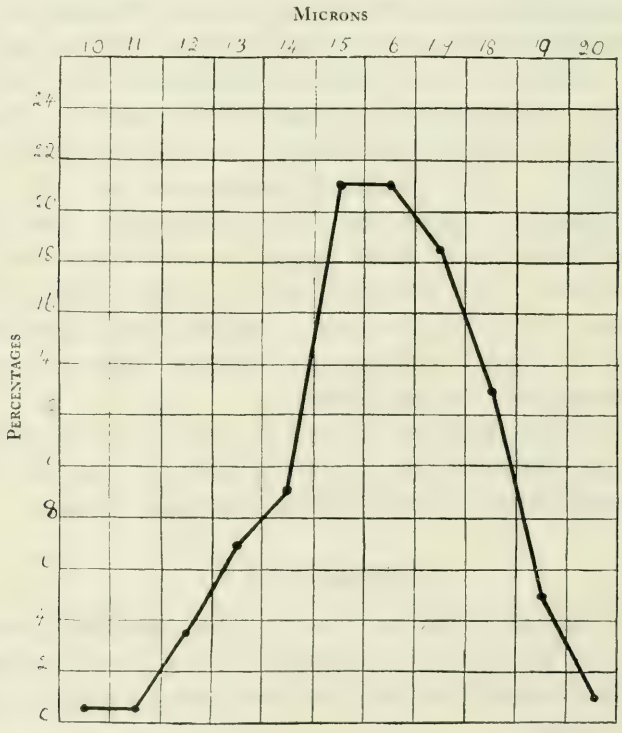


CHART 6.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma montgomeryi*.

TRANSMISSION

The vector of *T. montgomeryi* (?) is unknown.

DIAGNOSIS

It will be seen that, morphologically, this parasite differs widely from *T. pecorum*. The average length is greater, 15·88μ, as compared with 13·6μ. The most characteristic difference, however, is the great width of the organism, which is, on an average, 2·2 times that of *T. pecorum*: average breadth 3·29μ, as compared with 1·5μ. It appears to resemble most closely the parasite described by Montgomery and Kinghorn* as the Ninamwenda strain, and for which the name *Trypanosoma montgomeryi* was proposed by Laveran. But in view of the fact that we were able to examine material from one dog only, we cannot regard its identity with this parasite as established.

7. *TRYPANOSOMA IGNOTUM*, sp. nov. (Pl. XXI, figs. 1-8)

This parasite was obtained by feeding with *Glossina morsitans* on monkeys on ten occasions, and assuming that only one fly was infective in each instance, the proportion of infective to non-infective flies is 1 to 300, or 0·3 %.

MORPHOLOGY

(*a*) *Fresh preparations.* The parasite appears as a comparatively short slender organism. It is fairly actively motile, but exhibits no marked degree of transitory power.

(*b*) *Stained preparations.* The trypanosome is a slender organism of moderate length. The posterior extremity is obtuse, or bluntly rounded, while the anterior is effilated. The nucleus is rounded or oval, and lies at the middle of the body. The blepharoplast is small and rounded, and while usually situated near the posterior extremity, may be separated from it by an appreciable interval. It frequently projects from the edge of the parasite, forming a bud-like excresence. The undulating membrane is feebly developed, and a short free flagellum is only very occasionally present. As a rule, the cytoplasm shows no granules or vacuoles.

The average length of 500 individuals was 16·8μ, the maximum 23μ, and the minimum 12μ (see Table 42).

* Montgomery and Kinghorn, Annals of Trop. Med. and Parasit., Vol. III, No. 2, 1909.

TABLE 42.—Measurements of *T. ignotum*.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Goat 451	17	25	15·55	18·25	13·25
Monkey 1	8	25	16·67	20·25	13·5
,, ... 22	10	25	17·04	20·25	13·75
,, ... 22	11	25	17·99	20·0	15·5
,, ... 36	6	25	16·55	20·5	13·0
,, ... 230	9	25	17·38	23·0	13·5
,, ... 250	11	25	16·52	19·0	13·25
,, ... 363	9	25	17·1	20·0	12·0
,, ... 469	7	25	17·45	19·75	13·75
,, ... 469	8	25	17·19	19·5	13·0
,, ... 469	9	25	16·83	19·25	13·75
,, ... 518	12	25	16·99	19·0	14·75
,, ... 521	9	25	17·19	19·75	14·5
,, ... 521	10	25	16·87	20·25	14·5
Rabbit 44	37	25	16·9	19·25	13·75
,, ... 452	18	25	16·7	20·25	13·25
,, ... 452	20	25	15·43	19·5	12·5
,, ... 452	21	25	15·93	20·75	14·0
,, ... 497	13	25	16·88	21·0	14·0
,, ... 497	25	25	16·93	19·5	13·25
		500	16·8	23·0	12·0

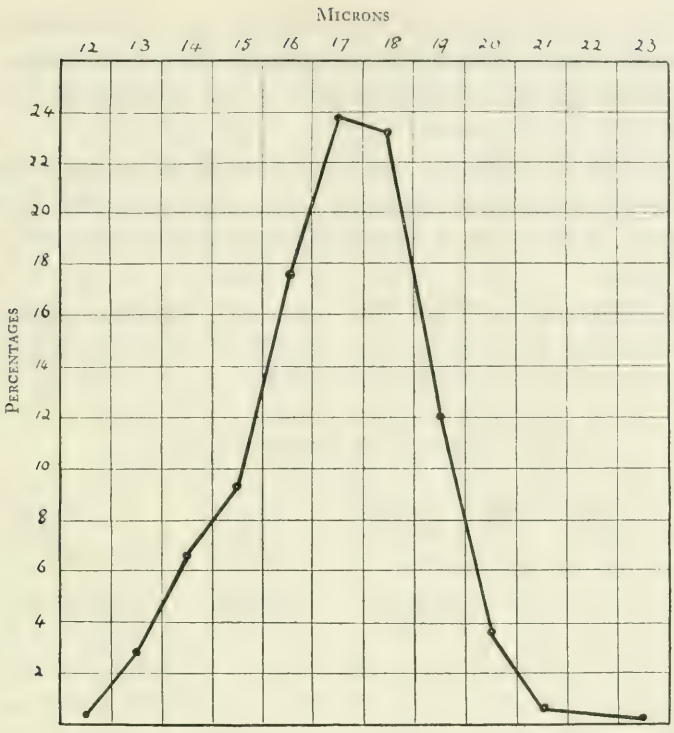


CHART 7.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma ignotum*.

PATHOGENICITY

In monkeys, the virulence of *Tryp. ignotum* was found to be very great. The disease is of a fulminating character, the parasites increasing rapidly in number until the animal's death. The trypanosome is of equal virulence in those animals infected directly by the bites of the tsetse flies and in those cases where the strain was passed from monkey to monkey. The incubation period varied from three to ten days, the average being seven, and death occurred two or three days after the parasites appeared in the peripheral blood.

It is interesting to note that on reaching the plateau three monkeys failed to become infected when inoculated with the valley strain of the parasite. At Nawalia, a failure was not recorded, and

it is difficult to understand those mentioned, more particularly as the parasite is of frequent occurrence in the plateau flies, and monkeys infected with this strain react in the same manner as those infected by the valley strain.

Three rabbits were successfully infected with the strain by sub-inoculations from a monkey. The incubation period varied from 13 to 22 days, and the duration of the disease 66 to 106 (still alive).

Two guinea-pigs, twelve rats, four mice, an ox, a goat and a dog were found to be refractory. Moreover, negative results were obtained by feeding infective flies on rats.

TABLE 43.—Pathogenicity of *Trypanosoma ignotum*.

Animal	Number used	Incubation days	Duration days	Remarks
Monkey	36	3—10 Average 7	5—16 Average 14	Three did not become infected.
Rat	12	—	—	None became infected.
Guinea-pig ...	2	—	—	Did not become infected.
Rabbit	3	13—22 Average 17	16—106	Two alive after over 100 days
Mouse	4	—	—	Did not become infected.
Ox	1	—	—	,, ,,
Goat	1	17	68 Still alive	Tryps. only seen once. Monkey inoculated on 55th day did not become infected.
Dog	1	—	—	Did not become infected.

DIAGNOSIS

Morphologically the parasite appears to be distinct from any hitherto described species. The graph (Chart 7) showing the distribution of the trypanosomes in respect of length resembles very closely that of *Trypanosoma uniforme*, but the parasites are at once distinguished by the fact that whereas *Trypanosoma uniforme* is

invariably furnished with a free flagellum,* this, as mentioned above, is of rare occurrence in *Trypanosoma ignotum*, sp. nov. Moreover, the difference between the two trypanosomes is clearly demonstrated by reaction of sub-inoculated animals. According to the Royal Society Commission, *Trypanosoma uniforme* is innocuous to monkeys, an observation which has been confirmed by Fraser and Duke,† who record that they were unable to infect these animals by sub-inoculation from game harbouring the parasite, although goats were readily infected. However, the fact that a large number of monkeys, and one rabbit, quickly succumbed to the disease indicates clearly that the two parasites are not identical.

No information is at present available regarding the alternative host of the trypanosome. Although the parasite has been isolated from wild *Glossina morsitans* much more frequently than any other trypanosome, it has never been found in game or domestic stock. Nothing resembling it has been seen in the peripheral blood of any animal examined in the Luangwa Valley and on the Congo-Zambesi watershed. These include 250 wild animals (elephant, rhinoceros, hippopotamus, buffalo, eland, zebra, wildebeest, roan, kudu, hartebeest, true waterbuck, Crawshay's waterbuck, puku, mpala, bushbuck, duiker, klipspringer, bushpig, warthog, lion, hunting dog, caracal, galago, squirrel, genet, giant rat and rabbit), 35 domestic animals (cattle, goats and dogs), 256 monkeys, 142 wild rats and 15 wild mice—making a total of 698. Eighty-six monkeys have been sub-inoculated from game and domestic animals, and in no instance has an infection with this trypanosome been observed.

In view of the fact that we have been unable to find the vertebrate host, we propose to name the parasite *Trypanosoma ignotum*.‡

*Reports of Sleeping Sickness Commission of the Royal Society, No. XI, 1911, pp. 160-164.

†Fraser, Capt. A. D., and Duke, H. C. Bull. S.S. Bureau, Vol. IV, No. 36, April, 1912, pp. 151-152.

‡ On returning to England at the conclusion of the work of the Commission, we found that the Royal Society Commission had published an account of a parasite which they called *T. simiae*. This is almost certainly identical with *T. ignotum*. As the paper of the Royal Society Commission appeared a few days before our paper on *T. ignotum*, this name must be dropped in favour of *T. simiae*, should the two eventually prove to be identical. (W.Y.)

DESCRIPTION OF PLATE XXI

Trypanosoma ignotum and *Trypanosoma montgomeryi.* Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-8. *T. ignotum.*

Figs. 9-16. *T. montgomeryi.*

TRANSMISSION

In one experiment the infective fly was determined to be one of a group of ten. These were then killed and dissected. Nine of the flies were found to show no trypanosomes in the gut, proboscis, salivary glands or sucking stomach, whereas in the tenth a heavy infection of the proboscis was encountered. The gut, salivary glands and sucking stomach were negative. This observation would indicate that the development of the trypanosome, *T. ignotum*, occurs in the proboscis.

8. *TRYPANOSOMA TRAGELAPHI*, sp. nov. (Pl. XXII)

This parasite was found in blood films made from a sitatunga (*Tragelaphus spekei*) shot near Mpika.

MORPHOLOGY

It is at once distinguished from all known mammalian trypanosomes, with the exception of *T. ingens*, to which it bears a very close general resemblance—so close, in fact, that in a previous report we referred to it by this name.

It is a long, fairly broad trypanosome with a well-marked undulating membrane, sometimes terminating in a short free flagellum. The posterior extremity is effilated. The nucleus appears as a broad band lying transversely across the centre portion of the body, and stains faintly with Giemsa. The blepharoplast is small but distinct, and is situated slightly posterior to the nucleus. The length of the five specimens seen in the single film examined were respectively 52·5, 53, 66, 70 and 72·5 microns, whilst the breadth at the level of the nucleus was 6, 8·5, 5, 6 and 7 microns.

DIAGNOSIS

Whether or not this parasite is *T. ingens*, we are, from the small amount of material available, unable to state with certainty. The general resemblance of the two trypanosomes is striking, but if the camera lucida drawings of this parasite be compared with those of *T. ingens* published in the report of the Royal Society Commission,

it will be seen that the trypanosome in question is shorter and more slender than *T. ingens*, and that the undulating membrane is broader and more pronounced. In the original description of *T. ingens* the length of five specimens are given as 72, 77, 82, 88 and 122 microns. The breadth is stated to be from 7 to 10 microns. It will be thus seen that *T. ingens* is on an average considerably longer than the parasite in question. The average length of the specimens of *T. ingens* measures 88 microns, whereas that of the specimens of this parasite is only 63 microns. We propose *T. tragelaphi* as a name for this parasite.

TRANSMISSION

Unknown.

This infected animal was one of a herd which lived in a large swamp formed by the Luitikila river, about four miles from Mpika.

The sitatunga only emerge from the swamp in the late evening and very early morning for an hour or two in order to feed, and even then do not wander more than a few hundred yards from the water.

Glossina morsitans have never been found within a radius of fifteen miles, and it is therefore exceedingly improbable that they are the vector.

Leeches are found in enormous numbers in the swamp, and feed voraciously on human beings. It seems possible that these animals are the vectors, more particularly as the parasite bears a very close resemblance to amphibian trypanosomes.

Mosquitos are also extremely plentiful in this swamp. Filaria were seen in the blood films of the same animal.

DESCRIPTION OF PLATE XXII

Trypanosoma tragelaphi. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of the camera lucida at a magnification of 2,000 diameters.

SECTION V

ON THE DEVELOPMENT OF *T. RHODESIENSE* IN *GLOSSINA MORSITANS*

BY

ALLAN KINGHORN, WARRINGTON YORKE

AND

LLEWELLYN LLOYD

In the course of our investigations, we endeavoured to accumulate information regarding the development of the human trypanosome in *G. morsitans*. Reference has already been made to this subject in a previous paper,* and it is here intended to correlate the facts at our disposal. It may be remarked at once that owing to the comparatively small number of laboratory-bred *G. morsitans* available, the information we have collected is by no means so definite as could have been desired.

Up to the present, comparatively little work has been done on this subject, and the records are more or less contradictory. Kleine,† who was the first investigator to write on the development of *T. gambiense* in *G. palpalis*, is of the opinion that the complete cycle takes place in, and is limited to, the intestine, whereas the Royal Society Commissioners‡ in Uganda consider that involvement of the salivary glands is essential. They state that without invasion of the salivary glands there is no infectivity of the fly.

TECHNIQUE

The method of dissection of the flies used by us was that described by one of us in a previous paper. Briefly, it consists in splitting the dorsum of the thorax longitudinally, and, after separating the muscles and loosening the tissues with needles,

* Kinghorn and Yorke. Annals of Tropical Med. and Parasitology, 1912, Vol. VI.

† Kleine. 'Trypanosomenstudien.' Arb. aus d. kaiserl. Gesundheitsamte, Bd. XXXI, Heft 2.

‡ Bruce, etc. Reports of the Roy. Soc. Commission in Uganda, 1911.

drawing out the salivary glands attached to the pharynx through the waist. This method has obvious advantages over that described by the Royal Society Commission, in which after snipping off the terminal segment of the abdomen the whole contents were expressed on to a glass slide, and the salivary glands subsequently separated from the mass of intestines and other structures. We claim for the technique adopted by us that the process is quicker, more certain, and that the danger of contamination from the intestines is reduced to a minimum. In fact, the only lesion in the alimentary canal accompanying the operation occurs in the anterior portion of the oesophagus.

To a certain point the information obtained from our dissections is exceedingly definite. We found that in every fly capable of infecting animals with the human trypanosome (*T. rhodesiense*) the salivary glands were invaded. Of the 160 laboratory-bred *Glossina morsitans* utilised in various experiments to transmit *T. rhodesiense*, 132 were dissected as they died. The remaining twenty-eight were too dry when discovered to allow of dissection. Twenty-seven of those dissected were found to be infected with trypanosomes. The day of the experiment on which the flies died and the results of dissection are given in Table 44.

A glance at the table shows that, of these 132 flies, five became capable of infecting animals with the human trypanosome. In each of these there was an enormous invasion of the salivary glands by trypanosomes. In the 127 flies which remained incapable of transmitting the parasite, the salivary glands were not involved, although trypanosomes were found in the intestines of twenty-two.

A precisely comparable state of affairs was observed on dissection of 'wild' *Glossina morsitans* which had become infective after feeding on infected animals. The salivary glands were found to be infected only in those insects which were capable of transmitting the human trypanosome. In all, 906 'wild' *Glossina morsitans* were used in these experiments, and of this number 620 were dissected. The remainder were for various reasons too dry to admit of dissection. Of these 620 flies the salivary glands of fourteen were invaded by trypanosomes. All except four of these were definitely proved to transmit the human trypanosome. In the case of the other four, the animal upon which the flies had been

TABLE 44.—Results of dissection of laboratory-bred *Gl. morsitans* which were found to contain parasites after being fed on infected animals.

No.	Date of infective feed	Day of dissection after infective feed	Results of Dissection			Remarks
			Proboscis	Intestine	Salivary gland	
1	9.9.11	4	O	+++	O	
2	10.8.12	4	O	+	O	
3	22.1.12	5	O	+	O	
4	9.8.12	5	O	++	O	
5	10.8.12	5	O	+++	O	
6	23.6.12	5	O	+	O	
7	8.8.12	6	O	+++	O	
8	9.8.12	6	O	+++	O	
9	8.8.12	7	O	+++	O	
10	23.6.12	7	O	+	O	
11	10.8.12	8	O	++	O	
12	8.8.12	9	O	+	O	
13	10.8.12	10	O	+++	O	
14	8.8.12	11	O	+++	O	
15	25.8.11	12	O	+++	O	
16	31.8.11	12	O	+++	O	
17	8.8.12	13	O	+++	O	
18	5.3.12	15	+	+++	O	
19	11.8.12	19	++	+++	+++	Infective on 12th day
20	10.8.12	20	+	+++	O	
21	9.8.12	21	2 tryps. seen	+++	O	
22	9.8.12	21	O	+++	O	
23	8.8.12	22	1 tryp. seen	+++	O	
24	8.8.12	22	+	+++	+++	Infective on 17th to 21st day
25	14.11.11	28	O	+++	+++	On 15th
26	22.1.12	29	O	+++	+++	On 19th
27	9.9.11	40	O	+++	+++	On 13th

N.B.—O = Negative, + = Scanty, ++ = Considerable numbers, +++ = Swarming.

allowed to feed died before a diagnosis could be made. None of the 606 flies in which the salivary glands were not involved were able to infect animals with the human trypanosome. Again, the infectivity of *Glossina morsitans* in nature was examined, as mentioned in a previous section, by feeding batches of freshly-caught flies on healthy monkeys. Certain of the groups infected monkeys, and from one of these infective groups the actual infective fly was isolated. This, on dissection, was found to have the salivary glands swarming with trypanosomes. The remaining 242 flies in this group, which had been shown to be non-infective when fed on monkeys, were dissected, and in no instance was an infection of the salivary glands observed.

TABLE 45.—Results of dissection of wild *Gl. morsitans* found to be capable of infecting animals with *T. rhodesiense*.

No.	Date of infective feed	Day of dissection after infective feed	Result of Dissection			Remarks
			Proboscis	Intestine	Salivary glands	
1	21.11.11	25	O	+++	+++	Infective on 11th day.
2	21.11.11	25	O	+++	+++	Infective on 11th day.
3	1.7.12	28	O	+++	+++	Infective on 13th day.
4	21.11.11	30	+	+++	+++	Not proved to be infective.
5	21.11.11	30	+	+++	+++	
6	21.11.11	30	O	+++	+++	
7	21.11.11	30	+	+++	+++	
8	14.2.12	39	O	+++	+++	Infective on 25th day.
9	4.10.11	40	O	+++	+++	Infective in nature.
10	30.6.12	42	O	+++	+++	Infective on 14th day.
11	30.6.12	47	O	+++	+++	Infective on 14th day.
12	13.6.12	58	O	+++	+++	Infective on 48th day after infected meal, or 8 days after having been placed in the incubator.
13	13.6.12	58	O	+++	+++	
14	13.6.12	59	O	+++	+++	
15	14.6.12	71	O	+++	+++	Not proved to be infective, but inoculation of trypanosomes from gut and salivary glands followed by positive results.

In all, twenty *Glossina morsitans* were found to have invasion of the salivary glands by trypanosomes, and of these, sixteen were definitely found to be capable of infecting animals with *T. rhodesiense*. Owing to unavoidable circumstances, we were unable to prove the point in the case of the remaining four, but there is no reason to doubt that had the animals on which these flies were fed survived beyond the necessary five or six days they would have proved to be infected.

In order to anticipate the criticism that the trypanosomes were not really inside the salivary glands, but lying outside these structures, and due to contamination from the gut, our examinations were conducted with extreme care. In the first place, the glands were removed uninjured and attached to the pharynx, placed on a microscope slide, and gently covered with a coverslip. By careful focussing, it could easily be decided that the parasites were actually in the lumen of the tubes and not outside. Moreover, they were usually present in such enormous numbers as absolutely to exclude the possibility that they were the result of contamination from the intestine. Again, the glands of other flies were removed with care and immediately fixed, and subsequently imbedded and cut. In the sections the parasites could be seen to be inside the glands. Finally, in order to remove any possibility of doubt, sections of the whole abdomen of these infective flies were made, and the glands found to be loaded with trypanosomes.

It will be seen from Table 44 that it was by no means a rare occurrence for trypanosomes to be present in the intestines in the earlier stages, especially in the case of those flies examined within a few days of the infected meal. As a general rule, however, most of the insects dissected after the first five or six days were negative. In a certain proportion multiplication of the parasites took place in the intestine.

As to the reason for this multiplication in the gut of occasional flies only, and as to the manner in which it occurs, we have obtained but little information. On one occasion a fly, which died on the 12th day after having been fed on a guinea-pig infected with *T. rhodesiense*, was found to have an enormous gut infection. Possibly there were also a very few trypanosomes in the salivary glands, but on this point we could not be absolutely certain, as the

insect had been dead for some time before the dissection was made. In the mid-gut were found a number of cysts containing swarms of trypanosomes. Some of the cysts had thin walls and were filled with a seething mass of flagellates, while others had thicker walls and the contents were quiescent. The cysts ranged in diameter from 27 to 32μ. Unfortunately, we are unable to state whether the fly was infective at the time of death. It had refused to feed for two or three days previously, and the animal on which it had last fed (9th day of the experiment) did not become infected. The gut contents were inoculated into a monkey, but the animal died from some unknown cause a couple of days later.

Although multiplication of the parasites occurred in the guts of a proportion of the flies, we met no instance in which a fly was infective and in which inoculation of the gut parasites into experimental animals gave rise to infection, unless there was an accompanying invasion of the salivary glands. On the other hand, it appears that on every occasion on which the salivary glands are involved the trypanosomes, both in these structures and also in the intestines, are virulent, i.e., the fly infects when fed on a healthy animal, and inoculation of the parasites from either the salivary glands or the intestine gives rise to infection.

The results of inoculation of trypanosomes from laboratory-bred flies in different stages of infection, and also from the wild flies which were proved to be infective, are given in Table 46.

Our knowledge of the manner in which the salivary glands become infected is uncertain, but there is a certain amount of evidence which would cause one to believe that it is secondary to the intestinal infection, and that it only occurs when the trypanosomes in the gut have reached a certain stage of development, and only then when the conditions of temperature are suitable for the further development of the parasites. In the first place, of 752 flies dissected at various intervals after having fed on infected animals, we never found trypanosomes in the salivary glands in the earlier stages before the flies were infective. Again, whenever trypanosomes were found in the salivary glands there were also enormous numbers present in the intestine. Moreover, it is significant that whenever trypanosomes were found in the salivary glands they were always infective, as were also those present in the gut.

No.	Result of feeding on normal animal the day before the fly was dissected	Day dissected after the infective feed	Proboscis	Intestine	Salivary glands	Result of inoculation of trypanosomes from different portions of the fly into clean monkeys or rats
1	Negative	4	O	+++	O	Gut contents; monkey not infected.
2	,,	5	O	+	O	,, ,,
3	,,	8	O	++	O	,, ,,
4	,,	9	O	+	O	,, ,,
5	,,	10	O	+++	O	,, ,,
6	,,	11	O	+++	O	,, ,,
7	,,	12	O	+++	O	,, ,,
8	,,	13	O	+++	O	,, ,,
9	Positive from 12th day onwards	19	++	+++	+++	Proboscis contents; monkey infected. Gut contents; rat infected. Salivary gland contents; monkey infected.
10	Negative	21	2 tryps. seen	+++	O	Gut contents; monkey not infected.
11	,,	21	O	+++	O	,, ,,
12	,,	22	1 tryp. seen	+++	O	,, ,,
13	Positive from 17th-21st day onwards	22	+	+++	+++	Gut contents; monkey infected. Salivary glands contents; monkey infected.
14	Positive from 15th day onwards	28	O	+++	+++	Gut contents; monkey infected. Salivary glands used for embedding.
15	Positive from 19th day onwards	29	O	+++	+++	Gut contents; monkey infected. Salivary gland contents; monkey infected.
16	Positive from 13th day onwards	29	O	+++	+++	Hind gut contents; rat infected. Fore gut contents; monkey infected. Salivary gland contents; rat infected.
17	Positive from 8th day after putting in incubator and 48th day after first infective feed onwards	58	O	+++	+++	Gut contents; rat infected. Salivary gland (right) contents; monkey infected. Salivary gland (left) contents; monkey infected.
18	?*	71	O	+++	+++	Gut contents; rat positive. Salivary gland contents; rat positive.

* Owing to the unfortunate death of the monkey on which this fly was fed we were unable to ascertain whether the insect was infective or not. The fly was one of the series which was kept for 60 days after the infective feed at laboratory temperature, and on the 61st day placed in the incubator. In view of the fact that the parasites in both the salivary glands and in the intestine were infective to subinoculated rats, it is highly probable that had the animal, on which the fly was fed, lived long enough, it would have been found to be infected.

Attention has already been drawn in a previous section to experiments which suggest that although the parasites can multiply and develop up to a certain stage in the intestine at comparatively low temperatures (55°-65° F.), yet the flies do not become infective until the temperature to which they are subjected is raised to at least 75°-80° F. In none of our experiments were trypanosomes found in the salivary glands of flies which had not been subjected to the higher temperatures. Probably the salivary glands become invaded by parasites which have reached a certain stage in their developmental cycle in the intestine. The remarkably short period (eight days or less) in which three flies, which had been kept forty days after the infective feed at laboratory temperature, became infected after being placed in the incubator at 85° F. can be best explained on the assumption that some portion of the cycle must have occurred in the gut during the first forty days at laboratory temperature.

Whilst the evidence that invasion of the salivary glands is secondary to that of the intestine is fairly conclusive, our knowledge of the path by which the parasites reach the glands from the intestine and of the morphological characteristics of the forms which migrate is by no means so definite.

It is at once apparent that there are two alternative routes by which the trypanosomes might reach the salivary glands from the gut, viz.:—(i) By way of the proboscis; (ii) by penetrating the gut wall, and after crossing the coelom traversing the wall of the salivary glands. We have accumulated no evidence enabling us to decide along which of these two routes the parasites migrate. Parasites were but rarely seen in the proboscis, and then, with one exception, only in small numbers. In the case referred to, trypanosomes were present in the proboscis in considerable numbers, but as both the gut and salivary glands contained enormous numbers of flagellates, their occurrence in the proboscis could easily be explained on the assumption that they had been discharged from the salivary glands and were passing down the proboscis with the secretion of these structures. The parasites from the gut, from the salivary glands, and from the proboscis were inoculated into monkeys and rats, all of which became infected, showing that the trypanosomes in each of these structures were virulent.

As will be seen from Plate XXIII, the parasites present in the gut and in the salivary glands of infective flies exhibit marked differences in morphological characteristics. The forms encountered in the intestine were many and diverse, but the predominant type was undoubtedly that depicted in figs. 7-12. It may be described as a large broad flagellate, with a feebly-developed undulating membrane and little or no free flagellum. The nucleus is usually compact and situated near the centre of the body, but not infrequently it lies in a more posterior position. The blepharoplast is, as a rule, situated near the posterior extremity, but sometimes lies further forward approaching the nucleus. In addition to these forms, long thin parasites were found. Here the nucleus was, as a rule, diffuse, and occupied a central position. The parasites stained more faintly, the undulating membrane was narrow, and there was generally a distinct free flagellum. Many more or less rounded bodies were also seen.

The form met with in the salivary glands of infective flies was almost invariably that represented by figs. 1-5. It approximates somewhat closely to the short form of the trypanosome in the blood of the vertebrate host, but is obviously not identical with this. The nucleus is compact, and is situated at the middle of the body; the blepharoplast is distinct, and lies near the posterior extremity. The undulating membrane is well developed; there is only occasionally a short free flagellum. The length of this form is 15-18μ. In addition to this form, long attenuated flagellates were occasionally seen, but they were very few in number, and it was necessary to search for a considerable period before discovering one of them. These forms resemble the corresponding variety seen in the gut. The nucleus is diffuse, and situated near the centre of the body. The parasite stains a faint pink with Giemsa.

In so far as we could determine, the predominant gut type was not met with in the salivary glands, and the predominant salivary gland type did not occur in the intestine. In spite of this, however, we are faced with the fact that the parasites in both situations proved to be virulent when inoculated into experimental animals. It appears to us that two hypotheses can be advanced to explain this; either that the salivary type is the only virulent form and that a certain number of these reach the intestine from the

salivary glands by way of the proboscis—this may be quite fortuitous, depending on the fact that a certain quantity of infected saliva is from time to time drawn into the intestine—and are then lost amongst the multitude of diverse gut forms, or that there is some unknown form (the one which migrates from the intestine to the salivary glands) which precedes the typical salivary gland type and which is also virulent for laboratory animals.

In conclusion, we might remark that invasion of the salivary glands was only observed in the case of flies infected with the human trypanosome (*T. rhodesiense*) and not in the case of any of the other trypanosomes with which we had to deal either in the Luangwa Valley or on the Congo-Zambesi watershed. This was the case both with the strain of *T. rhodesiense* derived from man and also with that found in 'wild' *Glossina morsitans* which had been infected in nature.

It is of interest to note that of 310 'wild' *Glossina morsitans* which were dissected as they were brought into the laboratory recognisable mammalian red corpuscles were found in the intestine of seventy, whilst nucleated red corpuscles were only found on twelve occasions.

SUMMARY

1. The salivary glands of all *Glossina morsitans* capable of transmitting *T. rhodesiense* are infected, and conversely without invasion of the salivary glands there is no infectivity of the fly.

2. Invasion of the salivary glands is secondary to that of the intestine.

3. The first portion of the developmental cycle of the trypanosome takes place in the gut. In order for its completion and for invasion of the salivary glands to occur, a relatively high mean temperature, 75°-85° F., is necessary.

4. Invasion of the salivary glands was only found in flies infected with the human trypanosome, *T. rhodesiense*.

5. The predominant type of the trypanosome in the intestine of infected *Glossina morsitans*—a large broad form—is quite

different from that which predominates in the salivary glands, where the parasite resembles somewhat the short form seen in the blood of the vertebrate host.

6. Both the intestinal forms and also those from the salivary glands of infective *Glossina morsitans* are virulent when inoculated into healthy animals.

DESCRIPTION OF PLATE XXIII

Trypanosoma rhodesiense in *Glossina morsitans*. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-5. Typical forms seen in the salivary glands.

Figs. 6-14. Intestinal forms.

SECTION VI

REPORT OF THE ENTOMOLOGIST,

LLEWELLYN LLOYD

(*a*) *GLOSSINA MORSITANS* IN THE LABORATORY

The breeding of these flies was carried out continuously from the middle of June, 1911, to the end of May, 1912. Kinghorn* has already outlined the method adopted, and has described the larva and pupa. The flies were fed on native fowls at Nawalia, except for a short period when goats were used. At Ngoa the flies were fed at first on fowls, but afterwards entirely upon goats, which died quickly under the repeated feedings, doubtless owing to the trypanosomes with which the flies were naturally infected. Fowls would appear to be unsuitable food, in spite of the fact that the flies feed much more readily upon them than upon mammals. Fowl blood in the 'sucking' stomachs of the flies forms large firm clots, and apparently blood in this condition cannot be utilised by the flies, as the clots have been found to persist for several weeks, monkeys having been used as blood donors in the interim. A fly with so large a clot in the body cannot retain a full-grown larva, and consequently many abort. The phenomenon is rare with mammalian blood, and it has not been observed in nature.

The bottles used for the stock flies were glass cylinders, five inches deep and two inches in diameter. At first only three flies, two females and a male, were kept in each tube, but it was found later that the number could be increased to six without damaging the vitality of the insects. In damp weather many flies die through fouling themselves with the copious semi-fluid faecal deposits. This may be obviated by inverting the tubes over sheets of blotting paper, which absorb the excreta, but precautions must then be taken to avoid the escape of the larvae. This was done by placing the tubes in dishes and paper-lined boxes. When flies are kept under these conditions the daily changing of the tubes becomes unnecessary—a great saving of labour when many flies are kept.

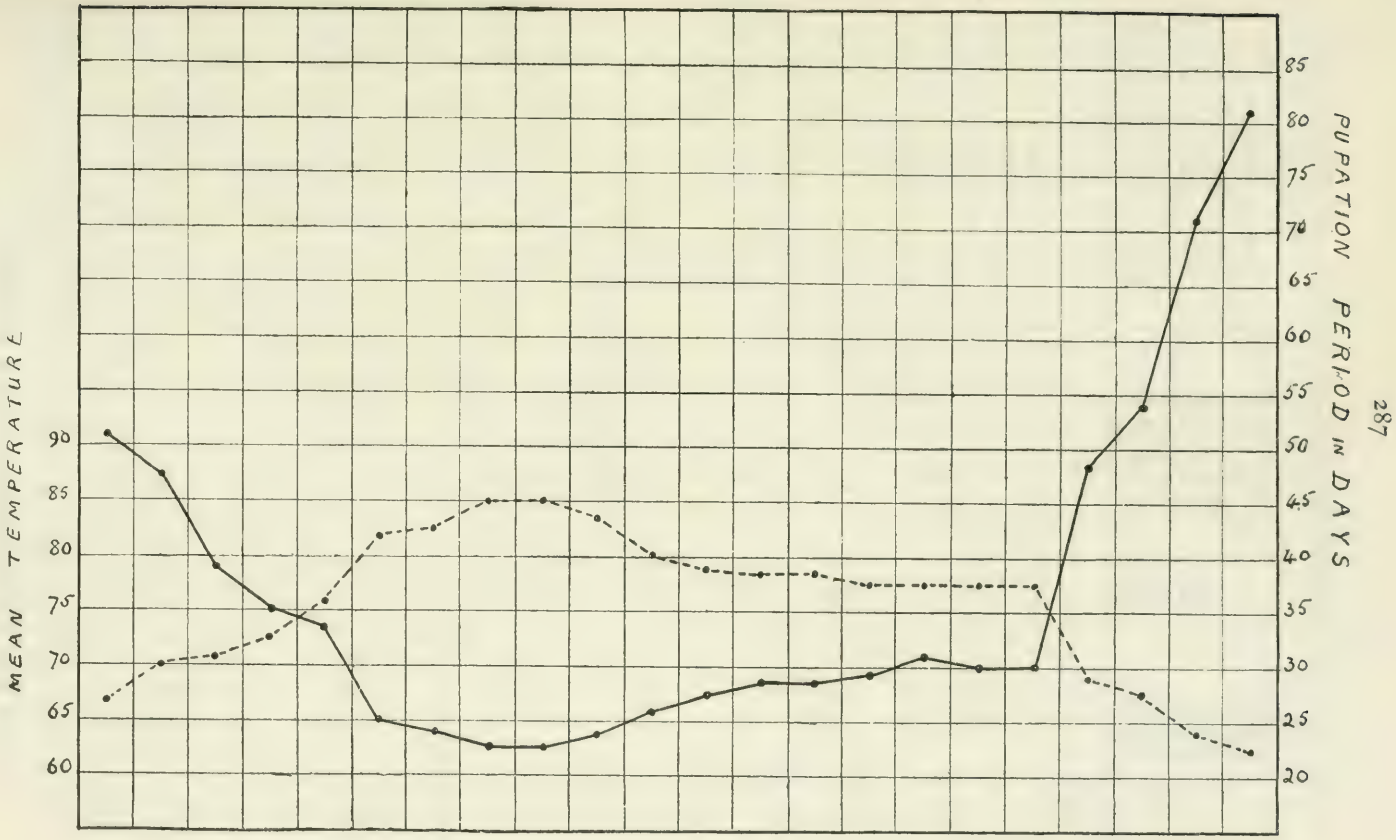
* Kinghorn, A. Bulletin Entomological Research, 1912.

It is sometimes difficult to obtain a sufficient supply of female flies, but natives learn to distinguish between the sexes and to select and bring in females. In a collection made without discrimination, the proportion of females is often as low as 2 %. During the twelve months the number of females in stock has varied considerably. From July to February, with the exception of a period at the end of August when all were destroyed by an accident, the number varied between 200 and 300. During April and May the number of females was between 500 and 600. The flies breed most readily at the beginning and at the end of the rains. This is confirmed by observations on the number of flies in nature at different periods of the year.

Males and females emerged from the pupae in approximately equal proportions for the twelve months. From June to December the males in each month were somewhat in excess of the females, while from January to April the reverse was the case. The pupation period of a male is on the average about a day longer than that of a female.

The variation in the duration of the pupation period is given in Table 47, p. 288. It shows more fully the influence of the climatic conditions on the pupal life; the pupae have been divided into groups which were deposited in periods of half months. For each of these periods the mean temperature in the laboratory and the relative humidity of the air are given. A fuller account of the meteorological conditions is given in a previous section of this report. The approximate mean temperatures to which the various groups of pupae were exposed have been calculated by averaging the mean temperatures for the periods in which the groups were deposited, and for those periods following which could have influenced the duration of the pupation period. Chart 8 indicates how readily the pupae respond to changes of temperature. The humidity of the air has little or no influence on the pupal life, as will be seen by comparing the data of June and July with those of the second half of March. While the relative humidity of the latter portion was 10 % higher than in the former, the temperature and the duration of the pupal life were approximately the same in each case. It should be mentioned that the observations from June to the middle of March were made at Nawalia (altitude 2,100 feet),

CHART 8.—Showing the influence of temperature on the pupation period of *Glossina morsitans*.



The pupation periods are represented by the continuous line, the temperatures by the broken line.

TABLE 47.—Showing the influence of Temperature on the Pupation Period of *Glossina morsitans*.

Period during which the pupae were obtained	Number of apparently healthy pupae	Number of pupae from which flies emerged	Average duration of pupation period—days	Mean laboratory temperature	Approximate mean temperature to which pupae were exposed	Relative humidity of air
June 26—30 ...	3	3	51	—	67° F.	48·6 %
July 1—15 ...	6	5	47	64·1°F.	70	—
,, 16—31 ..	24	18	39	68·3	71	45·7
August 1—15 ..	21	9	35	69·4	73	—
,, 16—31 ..	5	1	33	73·0	76	35·8
September 1—15 ..	2	2	25	72·7	82	—
,, 16—30 ..	14	12	24	80·7	83	31·5
October 1—15 ..	22	15	23	82·9	85	—
,, 16—31 ..	20	10	23	86·4	85	31·8
November 1—15 ...	17	6	24	86·0	83	—
,, 16—30 ...	25	16	26	81·5	80	41·1
December 1—15 ..	27	23	27	80·6	79	—
,, 16—31 ...	13	12	28	78·8	78	69·1
January 1—15 ..	20	16	28	79·0	78	—
,, 16—31 ..	31	28	29	78·0	77	77·7
February 1—15 ..	48	32	31	77·2	77	—
,, 16—29 ..	40	30	30	76·9	77	73·8
March 1—2 ..	11	8	30	78·0	77	—
,, 21—26 ..	4	3	48	75·9	69	62·5
April 1—15 ..	25	16	54	77·2	67	—
,, 16—30 ..	51	22	70	68·0	64	53·7
May 1—15 ...	71	20*	81	70·0	62	—
,, 16—31 ...	96	—	—	63·9	—	51·3
June 1—15 ..	—	—	—	61·8	—	—
,, 16—30 ..	—	—	—	56·9	—	53·0

* On August 4 the remaining pupae were placed in an incubator.

while those for the remainder of the time were made at Ngoa (altitude 4,400 feet).

The shortest pupation period was that of a pupa deposited in the middle of October, from which the fly emerged in twenty-one days. The mean temperature to which this pupa was exposed was slightly over 86° F. The longest period was eighty-eight days for a pupa obtained during May and exposed to a mean temperature of 62° F. Reference to the table will show that these extremely high and low temperatures have a very deleterious effect on the pupae, evinced by the high percentage from which flies do not emerge. Many of the flies which emerged after periods of over seventy days were malformed or weak, and did not survive well. The mean shade temperature of the outside air was always higher than that of the laboratory, and the majority of the pupae found in nature in July were placed in such positions that for several hours a day they would be exposed to the sun, except for a slight covering of soil. It is therefore probable that these very long pupation periods do not occur under natural conditions.

(*b*) A RECORD OF SOME BREEDING PLACES OF *GLOSSINA MORSITANS*

The pupae of *Glossina morsitans* were found in a variety of positions at Ngoa in July; all in the neighbourhood of two permanent streams, the Kanchibia and the Kalamba. At the time, the middle of the dry season, the leaves of the deciduous trees were falling rapidly, and dense shade was only to be found actually in the beds of the streams. The bush is of the nature of an open wood with a large variety of trees. The big trees grow, in general, right to the banks of the streams without intervening plains. Tsetse fly are very numerous in this area.

The following is a list of the positions in which pupae have been found:—

(1) Under a thick branch (native name Mutobo) running about half an inch above the surface of the soil, on gently sloping ground fifty yards from a stream, one living and one empty pupa case were found. They were close to the base of the branch, and were lightly covered with soil.

(2) A similar position under a branch of a tree (native name Muombo) twenty yards from the stream, five cases and numerous fragments were found among dead leaves and humus.

(3) In a hollow in the trunk of a tree (native name Mpasa) a few inches above the surface of the ground, ten yards from the stream. The bottom of the hollow measured about four inches by two, and was full of a firm clay impregnated with resinous matter. Ten pupae, nine cases and numerous fragments were taken from the crevices in the surface, several of them quite exposed.

This spot, visited a few days later, yielded another pupa. The sun shines into this hollow for several hours a day, striking the surface of the soil.

(4) In two large hollows (filled with an old termite nest much broken down) at the base of a tree of the same species on the bank of the stream. One of the hollows was well sheltered and received no sun. In this, fifty cases and numerous fragments were found, but no living pupae. The other hollow received the afternoon sun, and in it one living pupa and several cases were found. (Pl. XXIV, fig. 1).

(5) In a well-sheltered hollow between three main branches of a tree (native name Mupapa), containing dead leaves but no soil or humus, one pupa and eight cases occurred. This tree was on the bank of the stream, and the hollow was about two feet above the ground. (Pl. XXIV, fig. 2.)

(6) In a shallow cup full of soil and roots in a tree (native name Mutobo) close to a stream. This hollow was quite exposed to the sun. One empty case was taken.

(7) One pupa and two cases were found in a hollow full of insect droppings, two feet from the ground, in a tree (native name Muombo). The hollow was exposed to the sun, and the tree was two hundred yards from the stream.

(8) This position resembles the first two. One pupa and one case were found under the trunk of a tree (native name Muanga) which ran for six feet close to the ground before rising. The tree is four hundred yards from water. (Pl. XXV, fig. 3.)

(9) Three empty cases were found in two shallow cups, quite exposed to sun and rain, in a Mutobo tree. The cups contained a little dust and were four feet from the ground, being formed by a swelling on the trunk caused by the pollarding to which the

Awemba tribes subject the trees when making gardens. Three cases were found.

(10) A shallow in a Muombo tree, three feet from the ground, full of loose soil and roots and quite exposed. The tree was two hundred yards from water. Three pupae and five cases were taken.

(11) The stump of a rotten tree built around by termites, which still occupied the base. Twelve living pupae and many fragments were found under the branch and in and about the termite nest. The position was not shaded, and the stump would be well warmed by the sun.

(12) A main branch of a Mutondo tree had been cut across, the cut surface facing upwards. The wood had rotted away internally, forming a cup full of humus and exposed to the sun. Ten living pupae and four cases were found here. (Pl. XXV, fig. 4.)

(13) Two pupae and two cases were found in a hollow in an Mpasa tree two hundred yards from water. The hole was eighteen inches above the ground, and was full of insect droppings.

(14) In the deserted burrow of an ant-bear (*Orycteropus capensis*). The hollow had been occupied and enlarged by warthog during the last rainy season. The burrow ran in such a manner that the morning sun would shine in obliquely or to one side. On this side, and near the opening, nine living pupae and six empty cases were found, but no pupae were found on the sheltered side. In a similar burrow near, which was sheltered from the sun, no pupae were found. This locality was about three hundred yards from water. (Pl. XXVI, fig. 5.)

(15) In a burrow of an ant-bear half a mile from water one empty case was found.

(16) Two living pupae were found in a 'salt-lick' in the side of an ant-hill two hundred yards from water. The position was exposed to the sun. These 'salt-licks' are very common in the district, and are visited by most of the big mammals which scrape and lick away the soil for the sake of the salt it contains until large excavations are formed.

(17) A hollow one foot from the ground in a Musangati tree, full of hard clay and well sheltered, contained two cases.

(18) Ten cases were taken under the trunk of a Mpasa tree

running close to the ground. The position was well sheltered, and was twenty yards away from the water.

(19) A hollow between two main branches of a Chimpampa tree contained two cases. The hollow was filled with dead leaves and humus, and was well sheltered. The tree was close to water.

The pupae were thus found in association with trees of eight different species and in holes in the earth. The native names only of the trees can be given at present. All the trees about which they were found were either abnormal or injured. None have been found at the bases of normal trees or under bushes. The pupae were generally, though not always, hidden under slight covering of earth or dead leaves. These observations confirm those of Jack*, who first recorded the finding of the pupae in nature, that they are deposited in such positions that they are not likely to be scratched up by game birds.

The relation of the positions to shade is of interest, and is probably connected with the season. Of the fifty-four living pupae found, forty-nine (90 %) were so placed that they would be daily warmed by the sun. They were collected in the coldest part of the year, and at a time when flies were emerging from pupae in the laboratory after a period of from seventy-five to eighty-five days. The shade temperatures outside were at the time about 2° F. higher than those of the laboratory, so that in well-shaded positions the pupation period would be about seventy days. This protracted period would be much reduced in pupae which were exposed to the warming influence of the sun.

Reference to Table 47 giving the relation of the pupation period to the mean temperature will show that for temperatures below 70° F., an increase of 1° in the mean temperature causes a reduction in the pupation period of from three to five days. Several of these sunny positions would be waterlogged during rain, and therefore unsuitable for the deposition of pupae.

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* Jack, R. W. Bull. of Ent. Res., Vol. II, pp. 357-361.
Austen, E. E. A Handbook of the Tsetse Flies, London, 1911, p. 56.

(c) A RECORD OF BLOOD-SUCKING INSECTS AND TICKS COLLECTED IN THE LUANGWA VALLEY FROM AUGUST, 1911, TO MARCH, 1912, AND AT NGOA FROM MARCH TO MAY, 1912

Ixodoidea

Ornithodorus moubata, the Nkufu tick, was taken in three villages in the neighbourhood of Chinunda. In two instances it occurred in single huts in the villages in very large numbers, but was said not to have been seen in any other huts. In the third village it was found in two huts, in one of which it was numerous, while in the other, a deserted hut, a single specimen was taken. In this village the information was given that the pest had occurred in almost every hut, but had disappeared from all but the one. The absence of this pest from the Nawalia district is peculiar, as it must be constantly introduced from the higher ground where it is common. The natives attribute the absence to the heat, but a number of Nkufu brought to Nawalia from the above-mentioned villages lived and bred in the laboratory throughout the hot weather.

Tabanidae

These insects, which are very numerous both as regards species and individuals in the Luangwa Valley, were very troublesome in the laboratory, to which they were doubtless attracted by the numbers of animals about the place. Seventeen species of the genus *Tabanus* alone were taken in the laboratory at different times.

Chrysops. Only one species, *C. fuscipennis*, Ric., was seen, and a single specimen was taken at Nawalia in February. It flew out of long grass over swampy ground and settled on the hand. It was so eager to feed that it was readily caught in a tube.

Tabanus

Insects of this genus appeared spasmodically from August to October, the driest months of the year. In November, after the first showers of rain, they become much more numerous, and continued to appear in large numbers throughout the wet months, becoming scarcer again in March. In the latter month several species reappeared which had not been seen for some time, indicating that they were probably emerging from the pupae about this time. Males of several species were also taken in March. The following species were collected:—

T. africanus, Gray. This species was taken at intervals at Nawalia during the rainy season. It was most common during February and March, when it was frequently seen in the laboratory.

T. biguttatus, Wied. Only three specimens of this large species were seen. They were taken in the laboratory and in a canoe in November and February. The males, usually described as being commoner than the females, were not seen.

T. par., Walk. This was one of the most numerous species around Nawalia. It constantly entered the laboratory, and was most persistent in its biting. First seen in October, it became numerous in January, and its numbers declined towards the end of the rains.

T. liventipes, Surc. This somewhat rare species was taken in small numbers at Nawalia in January and February. Specimens were taken on kudu, on roan and about the camp.

T. nigrostriatus, Ric. Hitherto recorded only from Nyasaland and in small numbers, this species was one of the commonest and most troublesome at Nawalia. It entered the laboratory in large numbers, and was often seen on dead game during November and December. It gives very painful bites, settling especially on the head and biting through the hair.

T. taeniola, P. de B. This fly was taken first in October, and became common in November and December at Nawalia. It was again taken in some numbers in March. During the latter month a male was taken at Ngoa.

T. taeniola, var. *variatus*, Wlk. This variety was much more numerous than the typical species. It was first taken in September biting a native at sunset. It was not seen again till November, when it became very troublesome. During January and February it was not observed, but was again taken in some numbers in March.

T. fraternus, Macq. Never very common; specimens of this species were taken at Nawalia in August, November, December and February.

T. fuscipes, Ric. This species first appeared in January at Nawalia, and continued to be numerous to the middle of March. Both sexes were seen in large numbers resting on long grass over a swamp in the evening about the middle of January. In February and March about ninety female specimens of this fly were dissected in connection with the work of the Commission. The gradual maturing of the eggs was thus observed, and early in the latter month some had deposited part of their ova.

T. albipalpus, Wlk. A single female specimen of this species was taken at Ngoa in March. The species has hitherto been regarded as belonging to the West African Fauna, all the specimens having been taken on the West Coast with the exception of one which is recorded from Uganda.

T. pullulus, Acert. This species was taken at Nawalia in small numbers from January to March.

T. claritibialis, Ric. First taken in the laboratory at Nawalia in January. This species was fairly numerous in the two following months.

T. atrimanus, Lw. A single female of this fly was taken in the laboratory at Nawalia during October.

T. copemani, Aust. This species, which has been previously recorded from the Feiera district of Northern Rhodesia and from Nyasaland, was first taken at Nawalia about the middle of January, and in the two following months was one of the commoner species.

T. diversus, Ric. A female specimen of this species was taken in a tent at Kasongo on the Luangwa river in October. One other specimen was found dead on a table in the laboratory, at Nawalia in December. It had been caught by a spider, and was densely wrapped up in a web.

T. maculatissinus, Macq. One female of this was taken feeding on a rhinoceros at Ngoa in May. The distance from water was about a quarter of a mile, and the insect flew to the animal shortly after it had been shot.

In addition to the above, five other species of *Tabanus* were collected at Nawalia and Ngoa. One of these, I am informed, is an undescribed species, specimens of which have previously reached

the British Museum from various localities, and which will shortly be described. The remaining species have not yet been identified.

Haematopota

This genus was not nearly so conspicuous as the preceding one, either as regards species or individuals. One specimen was taken in December, and they were more numerous in January and February. After the latter month they were extremely scarce.

The following species have been identified:—

H. mactans, Aust. A single specimen was taken on a kudu in January at Nawalia.

H. insidiatrix, Aust. This species was several times taken in the laboratory at Nawalia during January and February, and it was also collected on antelope.

H. sp. An undescribed species which has previously been collected in Northern Rhodesia. Several were taken on antelope.

Muscidae. Glossina. The only species of this genus that has been collected is *Glossina morsitans*, Westwood. In the district to which the work of the Commission has been confined this insect is generally distributed. While small areas exist, such as the immediate neighbourhood of Mpika, in which the fly is not actually resident, there is probably no spot in which it does not occasionally occur.

Auchmeromyia luteola, F., is common both in the Luangwa Valley and on the Plateau. During September a large number of huts were searched, and one or more stages of this insect were seen in every hut. The adult insect is to be found throughout the year.

Cordylobia anthropophaga, Grunb. The larone of this fly were several times seen in the legs of wild rats. One larone which was squeezed out of its sac burrowed in sand and pupated, emerging as a fly after a very brief pupation period.

Stomoxys nigra, Macq. A few specimens of this species were collected at Nawalia in January. They were found resting on leaves and grass over a swampy stream.

Stomoxys calcitrans, Linn. (?) was very numerous at Mpika in March about the cattle kraals and on some dogs. In May, when some were required for experimental purposes, only one or two

individuals were found. At this time there had been no rain for two months.

Pupipara

Hippobosca hirsuta, Aust. This species was taken repeatedly on waterbuck (*Cobus ellipsiprymnus* and *C. defassa*), and on puku (*C. vardoni*), both in the Luangwa Valley and at Ngoa. On one occasion a specimen was taken on the back of a native who had been present when a waterbuck was shot a few hours earlier.

Two other species of the *Hippoboscidae* were collected at Nawalia. One of these, a wingless species, was taken on several occasions on bushbuck (*Tragelaphus scriptus*), while the other, a winged species, was only taken on one occasion on hartebeest (*Bubalis lichtensteini*).

My thanks are due to the Entomological Research Committee for the identification of many of the above insects. It would be difficult to exaggerate the usefulness of this institution to Entomologists in the field.

APPENDIX A

AN EXPERIMENT TO ASCERTAIN WHETHER TABANIDS TRANSMIT TRYPANOSOMES IN NATURE

BY

A. F. WALLACE, M.B.

M.O., N. RHODESIA.

AND

LLEWELLYN LLOYD

An experiment to determine whether flies of the genus *Tabanus* were vectors of trypanosomes in nature was carried out at Nawalia. As pressure of work prevented this being done at the time when Tabanids were most numerous, the number of flies used was small and the results are inconclusive. The experiment was carried out during February and March, the last two months of the wet season.

Each morning a number of fly boys were sent out to collect the flies, and brought in daily from ten to thirty, the number being larger when the sun was shining. These were placed in separate tubes, and were fed on a monkey. Only a small proportion of the insects placed on the animal took food. It seemed to make no difference whether the feeding was done in direct sunlight or in the shade.

All the flies that fed were dissected and examined the same day. The dissection of a *Tabanus* is a very simple matter. A needle is thrust through the head and the thorax is held with forceps. The fly is then immersed in salt solution, and a series of gentle pulls is given to the head. The chitin of the neck breaks across, and the alimentary canal is drawn out with the salivary glands. The gut breaks across in the weakest place, just in front of the proventriculus, and the head comes away with the salivary glands attached. To obtain the remainder of the gut in an unbroken condition, the last segment of the abdomen is cut round with

needles, and the gut then drawn out backwards. The proventriculus passes back readily through the thorax and brings with it the trilobed 'sucking stomach.' Microscope preparations were then made of the proboscis with the pharynx, the complete gut from proventriculus to anus, and the salivary glands. These preparations were examined for parasites.

In all, 128 flies were fed and dissected. These comprised the following species:—

T. fuscipes, Ric.	... ...	87 flies.
T. taeniola, P. de B.	... ...	25 ,,
T. claritibialis, Ric.	... ...	2 ,,
T. copemani, Aust.	... ...	10 ,,
T. pullulus, Aust.	... ...	2 ,,
T. par, Wlk.	... ...	2 ,,

These flies were fed between February 7th and March 15th. The monkey did not become infected.

Flagellated parasites were found in seven flies. In no cases were they seen in the proboscis or the salivary glands, but were confined to the mid- and hind-gut. Smears were made of the preparations, and in four instances inoculations were made into wild rats. Only two of the rats lived long enough for any conclusions to be drawn, and these remained negative.

APPENDIX B

AN ATTEMPT TO TRANSMIT *TRYPANOSOMA RHODESIENSE* BY MEANS OF *ORNITHODOROS MOUBATA*

BY

A. F. WALLACE

An attempt was made at Nawalia to transmit *T. rhodesiense* by *Ornithodoros moubata*. For this purpose ticks had to be brought down to Nawalia from the plateau at Mpika. It is a curious fact that there are no 'nkufu' (the native name for these ticks) in the Luangwa Valley, but that they abound in the hilly country on either side. This fact is well recognised by the natives, who explain it by the Luangwa Valley being too hot for ticks to live in. This may be the explanation of the high death rate in the experimental ticks.

Five experiments were conducted, about twenty-five ticks being used in each. Each group of ticks was fed on a monkey heavily infected with *T. rhodesiense*, and after an interval of about a month was fed on a clean monkey. Each lot of ticks was kept under observation for six to seven months, being fed at intervals on clean monkeys. Only a few of the ticks survived for seven months.

The following is a summary of the experiments:—

To begin with, 125 ticks were fed on a heavily-infected monkey.

After one month 87 ticks were fed on a clean monkey.

,, two months 51 ,, ,, ,, ,, ,,

,, three ,, 8 ,, ,, ,, ,, ,,

,, six ,, 23 ,, ,, ,, ,, ,,

,, seven ,, 10 ,, ,, ,, ,, ,,

The blood of the clean animals remained negative.

The result of interrupted feeding was also investigated. After the ticks had become partially distended with blood they were removed from the infected monkey and transferred to healthy animals. Certain of the ticks would not continue their meal, and in the case of those which did there was always a short interval, varying from a half to five minutes before they resumed. In all, sixty-one ticks were used in these experiments to ascertain whether the trypanosome could be transmitted from one infected animal to a healthy one by means of interrupted feeding. The result was negative in every instance.

ON THE NON-IDENTITY OF *TRYPANOSOMA BRUCEI* (PLIMMER AND BRADFORD, 1899) WITH THE TRYPANOSOME OF THE SAME NAME FROM THE UGANDA OX*

BY

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AND

B. BLACKLOCK, M.D., D.P.H.

(*Received for publication 7 December*, 1912)

INTRODUCTION

Before considering our own observations it will be necessary to review briefly previous statements regarding the morphology of *Trypanosoma brucei*.

(1) Bruce[1] states that the haematozoa vary among themselves a good deal in shape and size and seem to take on slightly different forms in different species of animals. He publishes four figures depicting nine trypanosomes. Possibly one or two of the five figured from the dog might be considered to be 'stumpy' forms.

(2) Kanthack, Durham, and Blandford[2] state that the Nagana parasites vary considerably both in size and form. They may be long and pointed and sometimes stouter, some individuals are short and thick with a short flagellum, their protoplasm being crowded with granules. This description suggests dimorphism, but it should be noted that forms without a free flagellum are not mentioned. No slides were available belonging to these observers, but Dr. Durham kindly lent us a large series of photographs. On examining these, one or, perhaps, two show a 'stumpy' form, but it is difficult to be certain, and the uniformity of the remainder is striking. They state that 'the material for our observation was obtained in the first instance from the blood of a dog infected by

* Read before the Royal Society of London on January 23, 1913, and reprinted from Proc. Roy. Soc., B, Vol. LXXXVI, pp. 187—191.

the disease on the voyage from Africa, and brought to England in November, 1896, by Dr. Waghorn.'

This animal we believe to be the origin of the strain of *T. brucei*, Plimmer and Bradford, 1899, described by these authors, and at present maintained in England, so far as we can gather, solely at Liverpool.

It is not stated above from what animal the dog was infected on the voyage, nor is it stated what the exact original source of the strain derived from Zululand was.

(3) Plimmer and Bradford[3, 4] describe four forms in the blood, but neither their description nor figures suggest that they have seen stumpy forms. They describe 'a large hyaline form.' 'This organism is much larger than the ordinary adult form, and is much wider, often more than double the width, and is more irregular in shape. The protoplasm is quite homogeneous and much more delicate, and it stains very faintly with the methylene blue.' They are still to be found in films, and we easily found them in Dr. Plimmer's old films, but we found only extremely rarely forms that could be called 'stumpy.' We think that if they had been present these observers would hardly have failed to have noticed and drawn attention to them, as they have a striking appearance.

(4) Bruce and others[5] make a comparison between *T. brucei*, Uganda, 1909, and *T. brucei*, Zululand, 1894.

They state that many of the old Zululand preparations are still extant, so that it has been possible to do this. The preparations were, however, 15 years old, and had been stained with carbol fuchsin. The slides were got from horse, donkey, ox, monkey, dog; 200 trypanosomes were measured from the Zululand strain and 172 from the Uganda strain. The curves obtained in this way certainly resemble one another, though in one case the peak is at 18μ, in the other case at 20μ.

Also trypanosomes are figured from each strain, and there can be little doubt that there is a close resemblance, if not identity, viz., in the fact that both possess both long and stumpy forms. 'With the evidence available the Commission consider themselves justified in considering the trypanosome recovered from the Uganda ox to be identical with *T. brucei*, the cause of Nagana in Zululand and other parts of South Africa.'

Further, Bruce states in another paper[6] that *T. brucei* (Uganda strain) has actually 26 per cent. of non-flagellated forms.

(5) In 1911 Laveran[7] published an article, entitled 'Identification et essai de classification des trypanosomes des mammifères.'

In this article he places *T. brucei* in his Group I, 'Trypanosomes chez lesquels le flagelle présente toujours une partie libre,' whereas he places *T. gambiense* in his Group III, 'Trypanosomes ayant des formes à flagelle libre et des formes sans flagelle libre.'

Or, in other words, *T. brucei* is classed among the monomorphic trypanosomes while *T. gambiense* is among the dimorphic. We have then two opposite statements as to the morphology of *T. brucei*, (1) that it is monomorphic and (2) that it is dimorphic.

We possess two strains of (so-called) *T. brucei* in the laboratory, viz., the Zululand strain, of which we have given the origin above, and the Uganda strain from Surgeon-General Bruce, obtained originally from the ox in Uganda in 1909. These strains have been maintained continuously at Runcorn in a variety of animals, the Zululand strain for $4\frac{1}{2}$ years, and the Uganda strain for $2\frac{1}{4}$ years. In the case of the Uganda strain it was lost in 1912 for a short period but was returned to us again by Prof. Mesnil, who had previously received it from us.

We made then a preliminary examination of these two strains, and found to our surprise that they could easily be distinguished morphologically.

We next proceeded to make a detailed examination of the two strains in a series of slides throughout the entire period of infection in various animals, viz., rats, guinea-pigs, and rabbits. As the result, we believe we have established the following facts:—

(*a*) The Zululand strain is *typically monomorphic*. The trypanosomes are long, with a long free flagellum. We must admit, however, that it is possible (as we believe is the case also in another typically monomorphic trypanosome, viz., *T. evansi*), to find by long search short forms which somewhat resemble true stumpy forms, but we must emphasise the fact that in all the slides we have examined, prolonged search is necessary to find them.

(*b*) We have also verified the fact that Laveran's *T. brucei* strain also is, as he says, monomorphic. The origin of this strain seems uncertain. Laveran probably received it from Ehrlich, but

where the latter got it from cannot now be ascertained. Unless it came from England, there must be two monomorphic *T. brucei* strains in existence, not to mention the possibility of other *T. brucei* strains of uncertain parentage in various laboratories.

We have examined also old slides from the Zululand strain lent us by Prof. Nuttall, Colonel Skinner, R.A.M.C., and Dr. Plimmer.* All these were monomorphic. We repeat here that in these films, or at least some of them, it was possible by long search to find a short form somewhat resembling a stumpy form, but not having the somewhat indefinable characteristic appearance of the latter.

(*c*) The Uganda strain, on the contrary, is *typically dimorphic*, i.e., besides the usual long forms of trypanosomes, stumpy forms are readily found, even in abundance occasionally, when the infection is well marked. Bruce,[6] as we have noted above, states that this trypanosome has 26 per cent. of non-flagellated forms.

The typical stumpy form we may define as a short, thick trypanosome, 12-14μ, almost straight or slightly curved along one edge, while along the other the membrane is thrown into bold folds, there being no free flagellum, or at times a very short or doubtfully free one.

It is thus easy to distinguish a typical Uganda specimen from a Zululand specimen, and, in fact, we may express the difference in this way, that it is impossible to match a typical Uganda slide by any slide from the Zululand strain.

We have stated in the above history of the Uganda strain that it was recently returned to us by Prof. Mesnil, who remarked in his letter that he had maintained it in mice (for nearly a year), and that it showed now very few 'trapues' forms. This we have been able to verify in the films made from the infected mouse sent to us. But, as soon as we had re-inoculated it into guinea-pigs, it again showed numerous stumpy forms. But the same does not hold good for the Zululand strain; in guinea-pigs, as in rats and rabbits, the strain is *typically monomorphic*, i.e., it does not show stumpy forms. We therefore conclude that the two strains, as we now possess them in the laboratory, are different.

* We desire here to express our thanks to these gentlemen for their kindness in sending us slides.

How, then, are we to explain these facts? There seem to us three possibilities:—

1. That the strain we now possess, which we have been designating *T. brucei*, Zululand, is not this strain at all, but some other trypanosome inoculated erroneously during the course of inoculations extending over years. We think this view is untenable, for it would not explain the monomorphic character of the old slides we have examined, nor would it explain Laveran's monomorphic trypanosome.

2. While Bruce may have been working with a dimorphic trypanosome in Zululand, and still has slides showing these characters, it is quite possible that the strain sent by him to England was something quite different. This is all the more likely, as Bruce successfully infected dogs from a variety of wild game, viz., wildebeeste, kudu, bush buck and buffalo, and, as Bruce himself states, 'when *T. brucei* was discovered in Zululand in 1894, it was naturally thought to be the one and only trypanosome in Africa,' and no suspicion arose at that time of a multiplicity of trypanosomes in native game.

This is the simplest explanation, and the fact that Plimmer and Bradford do not describe or figure stumpy forms, and our examination of Dr. Plimmer's slides had the same result, makes it probable that this is the true one.

3. That the strain originally sent to England was dimorphic, but that it has now become monomorphic. This may have come about in two ways:—

(*a*) The strain originally was a *mixture* of a long trypanosome and a stumpy trypanosome, and the stumpy has now died out. If this explanation were valid, it would probably imply that *T. gambiense* and other dimorphic trypanosomes were also mixtures. This we regard as a not impossible view, but one we cannot at present prove or disprove.

(*b*) The strain was originally *dimorphic* (but not a mixture), and that it has now become *monomorphic*. If this were so, it would modify materially our notions of specificity of trypanosomes, at least in laboratories. Of such a change we have at present not much evidence. We have noted, however, above that the Uganda strain

kept in mice for a year was almost (but not entirely) monomorphic, but that in guinea-pigs it at once showed its normal characters.

It is impossible at present to decide between these explanations.

We come back, therefore, to the fact of which we ourselves have no doubt, viz., that the trypanosome that Plimmer and Bradford worked with, and which they named *T. brucei* in 1899, is certainly now a monomorphic trypanosome, and is not the same as the trypanosome from the ox described under the same name by Bruce and others in Uganda.

We believe, then, that the facts we have brought forward prove the non-identity of the Zululand and Uganda strains.

In order to avoid confusion, we think it advisable that this Uganda trypanosome should be re-named. We therefore propose for it the name *T. ugandae.*

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CONCERNING THE SEX AND AGE OF AFRICANS SUFFERING FROM TRYPANOSOMIASIS

BY

JOHN L. TODD

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An analysis (Table II) of the 79 cases of trypanosomiasis seen among 12,298 natives of the Gambia Protectorate[1] shows that 76% of those found to be infected are adults, 24% of them are children and none are aged; there are more boys (16·4%) than girls (7·6%) among the cases, but the proportion of men and women infected is approximately equal. It is probable that these figures approximate a usual incidence of the disease in the Gambia since all of the cases seen there in 1902 occurred in children and middle-aged adults.[2]

An analysis (Table I) of the results obtained in 1911 by the examination of 9,069 Gambian natives, whose posterior cervical triangles were palpated for enlarged glands, shows that a slight degree of glandular enlargement is common among children of both sexes, but especially in boys—probably because boys are less careful of their person than are girls. Much enlarged '+' glands occur most often in young adults—76% of the cases of trypanosomiasis seen in the Gambia in 1911 belong to this class—and trypanosomes were found in every person, with much enlarged glands, who was examined for them.

Kinghorn[4] noted that all of 89 cases of trypanosomiasis seen by him on the Gold Coast were under 42 years of age; most of them were young adults. He also reported[5] that glandular enlargement is much more common in males than in females, and in children than in adults.

In Nyasaland, Sanderson[6] reported that of 47 cases of trypanosomiasis 38·3% were women and 53·2% were men,

TABLE I.—The incidence of enlarged posterior cervical glands among 9,069 natives of the Gambia classified according to their sex and age, 1911.

	Sex	Male			Female		
	Age	0—13	14—44	45—	0—11	12—39	40—
Percentages showing the incidence of varying degrees of glandular enlargement among members of the classes indicated	'+'* or much enlarged glands	0·4%	5·9%	0·2%	0·2%	6·5%	0%
	'+−' or slightly enlarged glands	2·2%	1%	0%	1·7%	1%	0·3%
	'+−−' or very slightly enlarged glands	42%	19·5%	11%	28%	13%	4%
	'−' or normal glands	55·4%	73·6%	88·8%	70·1%	79·5%	95·7%
	Totals ...	100%	100%	100%	100%	100%	100%
Number of individuals of each class examined	9,069	2,024	2,200	490	1,411	2,283	661
Percentage of total number of natives examined represented by each class	100%	22·3%	24·3%	5·4%	15·5%	25·2%	7·3%

* The precise degrees of enlargement signified by the terms '+,' '+−' and '+−−' are described on page 11 of our Congo report.[3]

8·5% were boys, while no girls were infected. Hearsey[7] also points out that, in Nyasaland, two cases are found among men for every one seen in a woman.

The observations mentioned in these reports have much in common. They all record the occurrence of more cases of trypanosomiasis among adults than among natives of any other class. Unfortunately, the ages of the natives examined and the degree of their glandular enlargements are not all recorded in the same way, so that it is not possible to compare these observations exactly with those made in the Gambia. But, because there is a general similarity between all of these observations, it seemed advisable to examine the records of natives seen in the Congo in 1903,[3] in order to ascertain whether the same conditions had existed there.

Almost 90% of the total number of cases of trypanosomiasis seen in the Congo Free State were in young adults (Table II). It was thought that these figures might give a wrong idea of the relative incidence of the disease at different ages, because, for two reasons, most of the natives examined were young adults; first, because natives of the Congo were unaccustomed to Europeans, and the tendency was for women, children and the aged to hide from the members of the expedition; and, second, because, by the opening up of the country, large numbers of young men, sometimes with their women, were drawn to the European settlements to act as labourers and soldiers—such persons were easily examined, and the cases of trypanosomiasis seen in European posts came almost entirely from among them. Consequently, the cases of trypanosomiasis seen in the Congo are divided (Table II) into two groups according as they occur in populations subjected to these selective influences or not. The total number of cases dealt with is comparatively small, but it is interesting, nevertheless, that the total percentage of young adults infected is the same in each group. Since the selected population contains many more males than females, it is only natural that the percentage of infected males is greater among cases of trypanosomiasis coming from it than among those drawn from a population not subjected to selective influences.

With the exception that the percentage of young people among the cases is greater, the incidence of trypanosomiasis in the Gambia differs slightly from its incidence in the Congo. The difference can

TABLE II.—The percentages of cases of trypanosomiasis seen in the Congo and in the Gambia, belonging to groups limited by sex and age.

Sex	Male			Female			Totals	
Age	0—13	14—44	45—	0—11	12—39	40—	Percentages	Number of cases seen
Cases seen among selected populations in the Congo, 1903-05	6·7%	63·2%	0·7%	2·6%	26·8%	0%	100%	269
Cases seen among unselected populations in the Congo, 1903-05	6·1%	57·2%	2%	2%	32·7%	0%	100%	147
Cases seen in the Gambia, 1911	16·4%	39·3%	0%	7·6%	36·7%	0%	100%	79

be accounted for, in great part at least, by the much larger proportion of adolescents among the Gambian natives examined; compare the figures, in Tables I and III, stating the percentages of the total number of natives examined, represented in each locality, by children, adults and old persons. In the Gambia, where the natives are habituated to the presence of Europeans, the huts were entered and, often, every member of a village was examined; but, even there, it is probable that many timid girls were not seen, while the large percentage of boys recorded is the result of their willingness to be examined. In order that it may be possible to compare the observations made in the Congo with those made in the Gambia, the figures for the Congo given in Table III, as far as possible, record only examinations made in native villages where no evident avoidance of the members of the expedition was manifest, and where the nature of the population was uninfluenced by economic conditions.

The results obtained by gland palpation in the Congo (Table III) agree with those obtained in the Gambia (Table I) in the general absence of enlarged glands in old persons; they are dissimilar in the larger percentages of children and of adults with considerable degrees ('+' and '+ −') of glandular enlargement. There is certainly a close connection between these increased percentages of enlarged glands and the much more frequent occurrence of trypanosomiasis among natives of the Congo; it has already been made clear that, in the Congo[3] as well as in the Gambia,[1] enlarged cervical glands mean trypanosomiasis. In 1911, it was estimated that 0·8% of the native population in the Gambia had trypanosomiasis; there, cases only occur sporadically.[1] It is scarcely possible to estimate the probable percentage infected among the natives examined in the Congo in 1903-05, but it is certain that 30%, and often more, of the natives in many of the villages visited were infected[8]; nothing approaching such an epidemic exists in the Gambia.

The smallness of the numbers recorded for old persons in all of the tables is striking. The figures given for the Gambia (Table I) are probably not very incorrect; though they doubtless tend to err, in the same way as those given for the Congo (Table III) which, for reasons already given, are probably lower

TABLE III.—The incidence of enlarged cervical glands among 4,647 natives of the Congo classified according to their sex and age, 1903-05.

	Sex	Male			Female		
	Age	0—13	14—44	45—	0—11	12—39	40—
Percentages showing the incidence of varying degrees of glandular enlargement among members of the classes indicated	'+'* or much enlarged glands	8%	8·4%	0%	6·2%	6·3%	0%
	'+−' or slightly enlarged glands	26·8%	24·7%	6·5%	23·7%	18·3%	5·5%
	'+−−' or very slightly enlarged glands	22·9%	24·7%	6·5%	22·4%	22·2%	27·8%
	'−' or normal glands	42·3%	42·2%	87%	47·7%	53·2%	66·7%
	Totals ...	100%	100%	100%	100%	100%	100%
Number of individuals of each class examined	4,647	586	2,073	15	193	1,762	18
Percentage of total number of natives examined represented by each class	100%	12·6%	44·6%	0·3%	4·2%	37·9%	0·4%

* See (3) p. 11.

than they should be for children, especially girls, and for the aged. There is no means of determining whether the numbers of individuals of each class examined approximate those normally existing in an African population, because vital statistics have not been compiled for that continent. But, it seems probable, from an inspection of statistics for European races, that fewer old persons and more boys were seen than might have been expected. In 1900, males over 45 constituted 9·9% of the population of Massachusetts, and there were 11% of females over 45; a cursory inspection of the Scotch and Irish census returns for 1911 suggests that these figures are, if anything, rather below those normal for a European population. The proportion of children, especially boys, recorded for the Gambia (Table III) is considerably larger than that in Massachusetts, where about 11% of the population were boys under 13 and 10% were girls under 11 in 1900; although as large a percentage of girls are recorded by the census of 1900 in parts of Scotland as were seen in the Gambia the percentage of boys there was about four points less than it was in the Gambia.

Table IV has been drawn up in order to ascertain how the percentages of natives, belonging to groups limited by sex and age characters, would vary in natives of the Congo living in districts infected or uninfected by trypanosomiasis. The natives recorded in it are taken from those whose glands were palpated in populations unsubjected to the selective influences mentioned above (Table II). Old persons are again strikingly absent among natives from both infected and uninfected districts. As might be expected, the proportion of adults, especially of men, is much less among natives of infected districts. The death rate among them from trypanosomiasis is greatest, because they are most exposed to infection by their occupations.[1] It is well-known and many observers have reported that fishermen and riverine tribes are especially liable to be infected by trypanosomiasis.[8 p. 27]

In appraising the value of the figures stated in this paper, it is necessary to consider both the methods employed in collecting the data on which the figures are based and the form in which they are presented. All of the natives considered in Tables I and III were apparently healthy persons, and the clinically recognisable cases of sleeping sickness, seen among these populations, are not

TABLE IV.—The percentages of natives, examined in infected and uninfected districts in the Congo, 1903-05, belonging to various groups limited by sex and age.

Origin of natives	Sex	Male			Female			Totals
	Age	0—13	14—44	45—	0—11	12—39	40—	
Natives from infected districts	Percentage of total number of persons examined represented by each class	17%	38·3%	0·5%	6·5%	37·2%	0·5%	100%
	Number of persons examined in each class	230	518	7	87	503	8	1,353
Natives from uninfected districts	Percentage of total number of persons examined represented by each class	9·5%	47·9%	0·06%	4·04%	38·5%	0%	100%
	Number of persons examined in each class	135	686	1	58	552	0	1,432

included in these tables. The age of the natives examined was roughly estimated by their appearance, and the degree of their glandular enlargement was ascertained by a single, rapidly-made examination; it is obvious that slight errors might easily occur both in the estimate of age and the statement of the degree of glandular enlargement; as has been described already, a proportion of the population examined certainly escaped observation. The age periods used in classifying the natives contain an unequal number of years and the periods used for males and females are not the same; these unusual age limits were chosen because they represent,[1] approximately, the periods during which male and female natives follow the occupations of children, adults and elderly people. Therefore, in comparing the figures given in these tables, it must be remembered that errors in compilation have occurred and that the span of years, allotted by the classification employed, is for adults almost thrice that apportioned to children; consequently, a meaning can be attached only to very considerable and constant differences in the figures stated for each class. Finally, the total number of infected and uninfected natives observed is too small; but in spite of this, and although the methods employed are not sufficiently accurate to permit far-reaching conclusions, it does seem as though something may be learned from a cautious consideration of the figures given in the tables accompanying this paper. It is believed that a part of the large percentage of adults with enlarged glands and a part of the large percentage of adults among cases of trypanosomiasis may be due to the greater number of years apportioned to the adult class, but it is also believed that most of both percentages is due to an increased incidence of trypanosomiasis among adults. That adults should be most infected is reasonable since their occupations—washing, drawing water, fishing, farming and travelling—expose them most to infection.

Trypanosomiasis, more prevalent in the Congo than in the Gambia, is doubtless one of the reasons why the proportion of old people is less among natives of the Congo than among those of the Gambia, and much less among both populations than might be expected. Even although a large proportion of adults infected with trypanosomiasis and untreated die within three or four years[9] after contracting their infection, and although the aged are not

exposed to infection by their occupation, it is astonishing that the percentage of old persons among the cases of sleeping sickness and the percentage of old persons with much enlarged glands should be so small; it seems impossible that even so small a number of persons could live during their years among populations so much infected with trypanosomiasis without having been infected with the disease themselves. The only explanation which suggests itself is that some natives may be immune to trypanosomiasis.[1, 2, 5, 10] If an immunity does exist there are many reasons and analogies to indicate that it is not an absolute, 'sterilizing' immunity but a relative one in which the host is tolerant of the infecting parasite. It is very much hoped that the natives, found to be infected in the Gambia in 1911, will be kept track of, as can be done easily by the Commissioners of the districts in which they were seen; a record of the course of the disease in them would add considerably to our knowledge of the duration and outcome of infection by *Trypanosoma gambiense* in Africans.

The conclusions concerning the natives of the Congo and of the Gambia, which can be drawn from the facts presented and discussed in this paper, are stated in the following paragraphs.

1. The proportion of elderly individuals among them is lower than it is among Europeans (Tables I, III, and IV).

2. By far, the majority of cases of trypanosomiasis are persons of middle age; almost none of them are elderly persons (Table II).

3. The percentage of individuals with a considerable degree of glandular enlargement—which is coincident with trypanosomiasis—is very much greater in adults, and in children, than in elderly persons (Tables I and III).

4. It is possible that the low incidence of trypanosomiasis among elderly persons may be due, in part at least, to an immunity acquired by them.

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THE IDENTIFICATION OF THE PATHOGENIC ENTAMOEBA OF PANAMA*

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INTRODUCTION

The identification of the entamoeba that causes dysentery and liver abscess in Panama has recently been placed on a satisfactory basis, since two of the members of the Hospital Staff have given special attention to the subject and have had referred to them for diagnosis all cases of entamoebic dysentery. Formerly each doctor diagnosed the cases in his own ward, and there was no uniformity in the identification of entamoeba, each man determining for himself whether in a given case the entamoeba was *E. coli* or *E. histolytica*.

Influenced by the work of Schaudinn and Craig, until a year ago I regarded all pathogenic forms as *E. histolytica*. In August, 1911, a careful study of the entamoebae found in clinical cases of entamoebic dysentery and entamoebic liver abscess was begun, and observations since then have led me to be of the opinion that the only pathogenic form in this region is *E. tetragena*, Viereck.

Having ample facilities for the collection of material from clinical cases, autopsies and the operating room, and for animal experimentation, attempts were made to determine the specific characters of the entamoebae so collected.

METHODS OF EXAMINATION

These were:—

1. Fresh and moist-chamber preparations and those stained intravitam with Gentian violet.

2. Wet-fixed preparations stained with haematoxylin, Romanowsky modifications and various other stains.

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3. Dry-fixed preparations stained by modifications of the Romanowsky method.

4. Animal inoculations and feeding experiments.

EXAMINATION OF FRESH PREPARATIONS

It was found that very few of the published descriptions of entamoebae were sufficiently helpful as guides for the purposes of identification, in fact every step required to be specially worked out, and clinical and morphological data correlated with animal experimentation.

Take the questions of refractility of the ectoplasm, and the colour of the cytoplasm, it seems to me that refractility is very largely a question of the age of the entamoebae or its relation to encystment without regard to species. I have found in cultures of free living forms that the younger amoebae were not very refractile, while those approaching the period of encystment were quite refractile. In a fatal case I found that large trophozoites deep in the submucosa in the floors of ulcers were very slightly refractile, while many of the smaller entamoebae in the more superficial sloughs were highly refractile, yet they were representatives of one species. In some clinical cases, all the forms seen were highly refractile, many contained chromidia and were the precursors of the small generation, from which the cysts develop. In other cases all the entamoebae were finely granular, contained no erythrocytes and were not refractile. I have never seen either with natural or artificial illumination the green or grey tints noted by several writers, and it does not seem to me that stained specimens bear out the notion that the green colour is due to lysed erythrocytes, for the latter are held intact for a considerable period in the endoplasm and disappear by condensation and erosion, yet retaining their staining characters. I should say that all observations were made with artificial light, transmitted through blue glass, and I wish to call attention to the necessity of using some such uniform method of illumination, for the difference in illumination between light derived from a blue sky or from white clouds makes their use as sources of light uncertain, and gives inconstant pictures usually not

noted by the observer. The resulting errors may be eliminated by the use of artificial light.

The nucleus is said to be inconspicuous in the pathogenic entamoebae in fresh preparations, but I have not always found this to be the case when artificial light has been used. It is true that in many individuals, or in all the individuals in some cases, the nucleus cannot be made out in fresh preparations. But when one learns to recognise the nucleus, nothing within the trophozoite is more conspicuous. The refractile granules or masses of peripheral chromatin stand out with great prominence, and may be followed with ease as the entamoeba moves.

The presence of an ectosarc is very constant in the trophozoites of the pathogenic form, and is a very important feature for differentiation from *E. coli*.

While in most cases it is true that all entamoebae detected in stools associated with pus, blood and mucus are pathogenic, it is not this type of case that presents difficulties. The puzzling cases are those in which entamoebae are detected in fluid stools containing mucus or in semi-solid or formed stools unassociated with blood or pus, but which it is necessary to diagnose at once so that treatment may be started energetically. As an aid to rapid diagnosis, I have used with some success gentian violet as an intravitam stain. The stain is used in the concentrated fluid form or diluted with an equal amount of water, and is either added to the flake of mucus or drawn under the coverslip and the preparation examined immediately. While many individuals become over-stained or remain under-stained, usually they will gradually take up the stain in such a manner that the peripheral chromatin, the centriole and karyosome in *E. tetragena* stand out with almost the clearness and definition of well-stained haematoxylin preparations fixed in the wet way, and differentiation from *E. coli* is easy. I have tried several basic stains, but none is so good as gentian violet for intravitam staining. Even when the trophozoite is very refractory to the stain, as in the small generation, and in the forms which contain chromidia, the nucleus for some reason will become more refractile and conspicuous. The non-refractile trophozoites take up the gentian violet rapidly, while cysts and the refractile forms take up the stain slowly.

EXAMINATION OF STAINED PREPARATIONS

Haematoxylin stained preparations were made from those fresh films which contained a sufficient number of entamoebae to warrant staining and study. The coverslip was removed and both slip and slide were fixed in Schaudinn's bichloride alcohol, or in Zenker's solution diluted one-fourth and one-eighth, as the full strength solution causes artefacts in the nucleus. Several haematoxylin stains were used, and particularly clear pictures were obtained with Mallory's phosphotungstic acid haematoxylin, with the modification that the films were not given a preliminary treatment with oxalic acid or permanganate but were placed over night in the haematoxylin and then differentiated with very dilute permanganate. This is a shorter way of using phosphotungstic acid haematoxylin and gives excellent results.

Fresh and wet-fixed preparations were controlled or compared with others stained after dry fixation by some modification of the Romanowsky stain. The films were thoroughly dried then stained with Hasting's stain, over-stained with Giemsa's stain, and differentiated with ammoniated alcohol. It was observed that the descriptions of pathogenic entamoebae, as well as figures used to illustrate them in the literature, did not correspond with the results obtained in well-differentiated films stained by the method just described. And it is believed that descriptions in the literature based on films stained in the usual way, that had not been sufficiently differentiated, are not as exact as they might be. Romanowsky stains have a tendency to over-stain just as haematoxylin does, though not so intensely as the latter, and this over-staining must be corrected by the use of a differentiating agent.

It has been my practice to stain in the following way. Fresh coverslip preparations containing a sufficient number of entamoebae to warrant staining and study, or those intended for diagnosis, are made into smears by sliding off the coverslip and thoroughly drying both slip and slide, after which each is stained with Hasting's stain for 15 minutes. Satisfactory films are then over-stained with Giemsa's stain until the film has a diffuse reddish purple tint. The film is then plunged into 60% ethyl alcohol containing about 1% of water of ammonia (10%), and differentiated in this,

washed in water, and controlled by the microscope until the purple substance of the nucleus and the blue colour of the cytoplasm are strongly contrasted. The film when properly differentiated has a blue-violet colour. If the film has been greatly over-stained it is treated with a momentary douche of 95% alcohol. Beautiful pictures are obtained in this way, but what is of more importance, the various figures displayed by the purple staining substance (karyosome?) can be noted and followed with ease. This purple staining substance in the nucleus of *Entamoeba tetragena* in dried-fixed films represents only a portion of the nucleus, as the centriole and peripheral chromatin do not stain purple by the above method. The purple staining substance in the nucleus of *E. tetragena* frequently appears as a ring, or as a reticulum or scattered granules. Its phases do not appear to have been accurately described, but have been confused with the appearances presented in wet-fixed films when basic stains have been used.

Here I wish to call attention to certain errors of interpretation which I believe have resulted from the failure to properly distinguish between the purple staining substance or karyosome of the nucleus in *Entamoeba tetragena* in dry-fixed films, and the nuclear substance which stains with basic stains in wet-fixed preparations. For example, figures showing the purple chromatin of the nucleus of *E. histolytica* represented by Craig and illustrated in its proper colour, have been used by other writers and figured in terms of haematoxylin or black and white, without explaining that the black in the figure represents the purple staining substance of dry-fixed Romanowsky preparations. Dry-fixed stained films should never be confused with those stained after wet fixation, for the pictures are different in each case, and the mistake should never be made of attempting to translate one into the other. In dry-fixed films the permeability of the entamoebae to stains apparently has been profoundly modified, for the centriole and the peripheral chromatin do not take or retain the purple stain. The karyosome alone retains the purple stain, while the remainder of the nucleus stains faintly blue, sometimes revealing the achromatic granules of uniform size which appear to form part of its structure.

FEEDING AND INOCULATION EXPERIMENTS

A number of kittens, cats, dogs and monkeys have been fed by mouth, or inoculated per rectum with cysts and trophozoites of *Entamoeba tetragena*. When tetragena cysts have been fed to half-grown cats there has resulted not the typical entamoebic colitis, such as is usually described in the literature, but an enteritis, *and in this lesion in the ileum trophozoites have been found which, though arising from tetragena cysts, had the morphology not only of E. tetragena but of E. histolytica and E. nipponica.* Now as a culture, which from its history and microscopical appearance was certainly a pure culture of *E. tetragena* cysts, was used in the experiment, and as the various forms appeared in the cat's intestines, I am led to believe that the trophozoites, described as *E. histolytica* and *E. nipponica* by various writers, are nothing more than the large trophozoites in the first place and atypical or degenerate forms of *E. tetragena* in the latter. I am confirmed in this opinion by never finding in my cases any of the perpetuating forms described by Schaudinn and Craig for *E. histolytica*. In one fatal case of tetragena infection, I observed from autopsy material many trophozoites that protruded pseudopodia not unlike those figured by Hartmann and Craig for *E. histolytica*. Their extremities were refractile, and appeared to contain a round spore-like body, yet, when these coverslip preparations were fixed immediately and stained with haematoxylin and by Romanowsky, the picture presented was that of *E. tetragena*, and the spore-like bodies had disappeared. In studying a strain of *E. tetragena* in cats, following rectal injection of trophozoites, I found at the fourth remove in dry-fixed preparations a great many trophozoites, the peripheries of which contained one or several lobose projections, the interior of which were very frequently deeply stained blue and suggested strongly the descriptions of *E. histolytica* by Craig. But these were artefacts, for in wet-fixed haematoxylin preparations from the same coverslip preparation the picture was that of *E. tetragena*, and associated with these trophozoites were several uninucleate tetragena cysts. If only the dry-fixed films had been studied in this case, they might easily have been described as *E. histolytica*.

With reference to rectal inoculation of trophozoites, infection practically always follows in animals of the right age, and death occurs within a few days (five to eight) if the strain is not too aged, while by mouth feeding the duration is longer (twelve days). In this, my experiments have not paralleled those of other writers. It is difficult to explain this. I may say that after several unsuccessful attempts to infect very young kittens and adult cats, I subsequently only used kittens weighing about 700 grams.

Animal experimentation is of very great value in studying the variations in a given strain. In a strain recently I have been able to watch senility gradually developing from week to week, the entamoebae becoming reduced in size and filled with chromidia, and ultimately becoming encysted.

The comparative study of staining and fixing agents has brought out some very interesting information. The very marked differences presented by dry-fixed and wet-fixed stained preparations have been referred to. Phosphotungstic acid haematoxylin gives varying pictures depending on the fixative. More information of the structure of the nucleus is obtained by the use of this stain after fixing with diluted Zenker's fluid (1/8 and 1/4) and Flemming's fluid. The karysome is not so well transfixed when treated with Merkel's, Hermann's or Schaudinn's fluid. In wet-fixed films stained by Romanowsky it was never possible to stain the trophozoites exactly like the tissue cells near by. When stained by Giemsa's method and differentiated in acetone xylol the peripheral chromatin usually stained blue, while the centriole and nuclear sap stained pink or red, the tissue cells near by displayed purple staining substance throughout the nucleus. It was possible in several cases to study first the tissue-destroying trophozoites, later the small generation, and finally during convalescence and after apparent recovery the cysts, although in practically all cases that received energetic medication the small generation and cysts did not appear.

The detection of cysts in convalescent and recovered cases is most important, for it is the cyst, and not the trophozoite, which is the infecting agent and makes the host of the former a 'carrier.' The identification of tetragena cysts is usually easy in stained preparations and should present no difficulties in fresh films, yet

the cysts are so small (12 to 15μ in diameter) that they have frequently been mistaken for monad or coli cysts or fat droplets, or possibly mononuclear leucocytes. When in doubt and the number of cysts was to small to risk loss by fixation and staining, I have vaselined the preparation or kept it in a moist chamber, and if the cyst was homogeneous at first, after one or two days, one, two or four nuclei became distinctly visible. Tetragena cysts are more commonly detected in neglected cases which have partly recovered from entamoebic dysentery who may have diarrhoea or whose stools are solid, and I have found cysts in a case that had been insufficiently treated by means of rectal injections. It would seem, however, that if a case of dysentery is treated early and energetically the trophozoites are at once driven from the field, leaving none to develop into the drug-resistant small generation from which the cysts arise. This, it will be seen, is analogous to the rational treatment of malaria, in which the asexual generation is destroyed at once by large doses of quinine, thus destroying all the forms from which the gametes arise.

In acute or new infections, or in very active lesions, many of the trophozoites are of large size—30 to 60μ in diameter. As the strain grows older, the size often becomes reduced, the trophozoites measuring from 12 to 24μ in diameter. These forms frequently contain coarse blocks of chromidia, and they constitute the 'small generation.' During convalescence and after apparent recovery there appear small trophozoites 12 to 15μ in diameter, and associated with them are cysts and four-nucleated schizonts. If relapse occurs with symptoms of colitis, large trophozoites make their appearance again, and the cysts will have disappeared. Thus, during the progress of a case of tetragena dysentery, at first large trophozoites will be seen, many or all of them having the characters described for *E. histolytica* by Schaudinn and Craig, and later if the case has been a neglected one, the small generation with cysts will be found, of which there is no better description extant than that in Elmassian's paper on this form, which he has called *E. minuta.*

CONCLUSIONS

If there is a sufficient number of cysts they may be fed by mouth to young cats, and it may be possible to recover, from their bowel lesions, trophozoites having not only the characters of *E. tetragena*, but of *E. histolytica* and *E. nipponica* as well. Or, if in any given case of tetragena dysentery, in which the trophozoites are of the histolytica type, they be injected rectally into a young cat and the strain carried on by subsequent rectal inoculation into other cats at the time the infected animal dies, so as to prolong the vegetative phase of division, then it will be seen that the *histolytica*-like trophozoites become reduced in size, filled with chromidia, and at the fourth or fifth remove it is possible to find uninucleate tetragena cysts. The nucleus of the trophozoites meanwhile has taken on a typical tetragena appearance with a prominent karyosome. It is now impossible to infect other cats per rectum with this material. If we make dry-fixed Romanowsky stained smears of material containing these typical tetragena trophozoites, they will occasionally present the morphological peculiarities of '*E. histolytica*' described by Craig. We are thus able to correlate most of the observations of Schaudinn, Craig, Elmassian and Koidzumi, and state that there is but one pathogenic entamoeba, and that one is *E. tetragena*.

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A NEW TSETSE FLY FROM THE CONGO FREE STATE; AND THE OCCURRENCE OF *GLOSSINA AUSTENI* IN GERMAN EAST AFRICA

BY

PROFESSOR R. NEWSTEAD, F.R.S.,

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(*Received for publication* 3 *April*, 1913)

Recently Monsieur G. Severin, of the Musée Royal d'Histoire Naturelle de Belgique, had the kindness to send me an extensive collection of tsetse-flies for identification, numbering in all nearly one thousand specimens, all of which had been collected in various parts of the Congo Free State. Among these were found two examples of a species which has proved to be new, and hitherto undescribed. I wish here to express my indebtedness to Monsieur Severin for entrusting me with the examination of the collection in question, and also to add that it affords me infinite pleasure to be allowed to dedicate this new species of *Glossina* to so distinguished a naturalist.

Glossina severini, sp. n.

MALE. Hind tarsi either uniformly dark brown or with the first and second segments slightly paler than the rest; pleurae and hind coxae dusky grey, harpes (fig. 1 *h*) *very small, narrow and irregularly serrated distally.*

Length (two specimens) 10·3 to 10·5 mm. Length of wing 10·5 mm.

Palpi relatively long and stout. Third segment of the antennae clothed with short hair. Thorax very robust, markings well defined and of the usual type or similar to those in *Glossina palpalis;* pleurae dusky grey. Legs with the hind coxae grey or greyish buff; tips of front and middle tarsi black; hind tarsi all dark (almost uniformly so in one example), or with the first and second segment slightly paler than the rest. Abdomen almost unicolourous. Genital armature (fig. 1)

with the superior claspers free, the hairs with which these appendages are clothed very long. Editum with very long marginal hairs, the longest reaching almost to the tip of the vesica (*v*); median process (*mp*) narrow, being much less than the width of the inferior claspers (*i. c.*) and projecting slightly beyond the latter; harpes (*h*) rudimentary, being quite short and narrow, with the distal margin irregularly serrated.

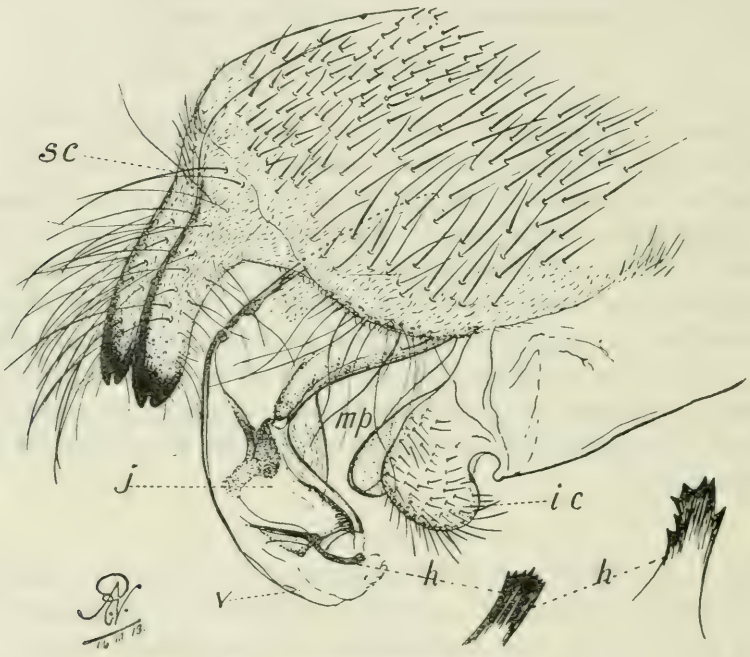

Fig. 1. *Glossina severini*, Newstead.

The type specimen of this species is a male in the Musée Royal d'Histoire Naturelle de Belgique. It was taken in the Congo Free State, and bears the following data:—'Lac Mouro (Riv. La Lavua), Mai, 1907.' It had evidently been preserved in alcohol, so that it is highly probable that the colours may have been slightly changed; but on comparing it with a number of examples of *Glossina fuscipleuris*, Aust., from the Congo Free State, and also some specimens of *Glossina fusca*, Walk., all of which had been preserved in the same way, one could readily separate both

G. severini, n.sp., and *G. fuscipleuris* from *G. fusca* by the much darker colour of the pleurae and the hind coxae.

A second example from the same Institution as the type bore the label 'Congo' only.

As to the affinities and characteristics of this new tsetse-fly, one gathers from the two examples that, so far as the colour of the hind coxae and pleurae are concerned, it resembles *G. fuscipleuris;* but it has relatively longer and stouter palpi; and furthermore is distinguishable from the latter by the darker and more uniformly coloured hind tarsi.

If we take the structural characters of the male genital armature, we find that it is very closely related to *G. medicorum*, Aust., from which it may, however, be readily separated by the curious rudimentary harpes, the greater length of the marginal hairs of the editum, and furthermore by the greater length of the lateral branches of the hairs of the arista (fig. 2 *a*).

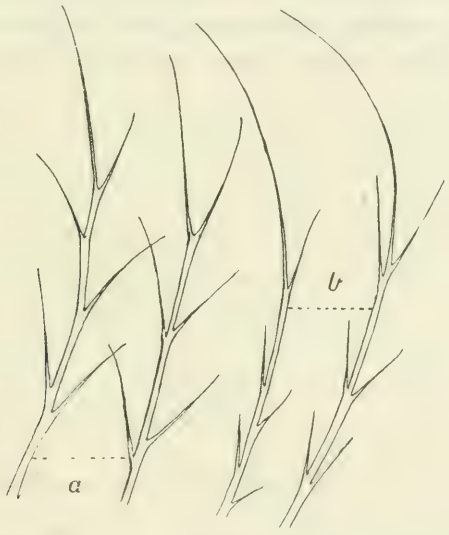

Fig. 2. (A) *Glossina severini*, n. sp. Terminal portions of the hairs of the arista.
(B) *Glossina medicorum*, Austen. Terminal portions of the hairs of the arista.

Soon it is hoped that perfectly fresh material will be available for a more complete study of the colour and pattern of this new

tsetse-fly, as too much importance cannot be attached to the colour in the existing specimens, owing to the fact that changes, as already stated, may have taken place. However this may be, the structural characters as set forth in the above diagnosis will very readily assist in the future determination of this interesting addition to the genus *Glossina*.

Glossina austeni, Newstead.

Twelve examples of this recently discovered tsetse-fly, consisting of two males and ten females, were submitted to me for identification by Dr. Hermann Morstatt, Amani. The examples in question were captured at Nyussi, Tanga district, January and February, 1913.

Though this is the first recorded instance of the occurrence of this species in German East Africa, its occurrence there is not surprising seeing that it has also been recently recorded from Portuguese East Africa. Its northernmost range, so far as is known at present, is Jubaland. It is evident, therefore, that this insect has a wide geographical distribution, and it is remarkable that so distinctive a species should have escaped detection until quite recently.

EXAMINATION OF THE ROOT OF AN *IPOMOEA* FROM RHODESIA

BY

PROSPER H. MARSDEN

LECTURER IN MATERIA MEDICA AND PHARMACY IN THE UNIVERSITY OF LIVERPOOL

(*Received for publication* 16 *March*, 1913)

PLATE XXVII

I am indebted for the specimen of the root of *Ipomoea* to Dr. W. Yorke, who brought it from Rhodesia. Dr. Yorke says of the root: 'It is the native remedy for a disease known as Chilatera, which has quite erroneously been confounded with Sleeping Sickness. A native doctor who visited our Sleeping Sickness Camp in the Luangwa Valley stated that several of the patients were suffering from Chilatera, and that he could cure them by administering this drug. On obtaining our consent he made a decoction of the root and administered it to the patients in question. Within an hour they all exhibited marked signs of gastro-intestinal irritation with acute vomiting and diarrhoea. I think that, as the drug has such a powerful cathartic and emetic action, it would be of interest to attempt to ascertain the active principle.'

The material consisted of a rough tuberous root (Pl. XXVII) weighing 260 grammes, 20 cm. long and 24 cm. in diameter, having the external characters of that of *Ipomoea horsfalliae* described by Power and Rogerson.*

MACROSCOPIC AND MICROSCOPIC CHARACTERS OF ROOT

The root was found to consist of a hard outer portion, of an ashy-grey colour, with warts upon it and exhibiting dark spots of exuded resin. The inner portion, evidently decomposed, showed a black pulverulent mass which was interspersed with greyish vessels.

* Wellcome Laboratories Report No. 171, reprinted from the American Journal of Pharmacy, August, 1910.

Upon examining the latter material under the microscope the vessels were seen to have thickened walls, and starch grains, simple and compound, were present in large quantity. The outer portion consisted of some ten layers of yellowish-brown cork cells, and resin-secreting cells were seen in the cortex and the bast, whilst starch grains were numerous in the parenchyma.

PRELIMINARY EXAMINATION

Upon digesting a small quantity of the inner portion of the root with water, in a water bath, for two hours an extract was obtained which was alkaline to litmus, gave no reaction for tannin or alkaloids, but reduced Fehling's solution, and gave a slight cloud with solution of acetate of lead, and a persistent froth upon shaking. An extract of the outer portion, prepared in a similar way, was acid to litmus, reduced Fehling's solution, and frothed upon shaking.

ESTIMATION OF MOISTURE

The moisture, estimated upon 14·29 grammes of whole root, was found to be 61·47 per cent.

EXAMINATION FOR VOLATILE OR FIXED OIL

As the root had a slightly aromatic odour it might contain a volatile oil; with a view of ascertaining this, 4·2 grammes of the finely-powered root was mixed with twice its weight of fine sand and exhausted in a Soxhlet apparatus and reflux condenser with petroleum spirit. Upon evaporation of the latter a syrupy liquid was obtained corresponding to 2·59 per cent. of the material taken. This quantity was too small to make much of, and upon passing steam over it in a closed vessel no aromatic odour was obtained.

EXAMINATION FOR PRESENCE OF RESIN

60 grammes of the dried root was cut up, and separated into outer and inner portions. These were present in the proportion of three of inner to one of outer. Each was powdered, mixed with twice its weight of fine sand and exhausted with hot alcohol in a Soxhlet apparatus. Most of the alcohol was recovered by distillation, and the remainder evaporated until the extract ceased

to lose weight. In the case of the outer portion the result was a soft extract corresponding to 6·95 per cent. of the material taken, and having an odour of extract of gentian. This extract was digested with water on a water bath, cooled and filtered; the filtrate was slightly acid to litmus and gave no reaction for tannin or alkaloids, nor did it reduce Fehling's solution. A dark green resin was left upon the filter paper corresponding to 0·85 per cent. of the dried outer portion of the root taken.

The inner portion of the root treated in a similar way yielded 8·19 per cent. of soft extract and 3·70 per cent. of resin.

I am much indebted to Messrs. G. B. Westmacott and Son, who very kindly procured from Jamaica for me a fresh root of *Ipomoea horsfalliae* for purposes of comparison.

Dr. Yorke is endeavouring to procure leaves and flowers of the plant which yields the root I have described above; until these arrive the identity of the drug must remain uncertain.

EXPLANATION OF PLATE XXVII

Tuberous root of *Ipomoea horsfalliae*—a native remedy for the disease known as Chilatera, in Rhodesia.

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OF
TROPICAL MEDICINE AND PARASITOLOGY

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The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1913:—

Full Course begins 6 January. Advanced Course begins 2 June.
Diploma Examination, 7 April. Certificate Examination, 30 June.
Full Course begins 15 September.
Diploma Examination, 15 December.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

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EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is Part A of the third number of the seventh volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

ON THE MORPHOLOGY OF THE TRYPANOSOME (*T. NIGERIENSE*, N. SP.) FROM A CASE OF SLEEPING SICKNESS FROM EKET, SOUTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A., B.Sc.

MEDICAL RESEARCH INSTITUTE, LAGOS, SOUTHERN NIGERIA

(*Received for publication* 25 *July*, 1913)

Early in 1912 attention was drawn to the presence of a disease resembling sleeping sickness in the Eket district of Southern Nigeria. Mr. S. A. Bill, of the Qua Ibo Mission, reported in February that a girl at a village called Ikot Offiong appeared to be suffering from trypanosomiasis. A medical officer (Dr. R. W. Gray) was accordingly despatched to the district to investigate this case, and to enquire into the presence of sleeping sickness in the neighbourhood of Eket. In May he was able to report that he had discovered two cases; and, as on further investigation the disease was found to be widespread, an isolation camp was established near Ikorobo. Up to the end of April, 1913, a total of 167 cases of sleeping sickness had been identified.

Sporadic cases of sleeping sickness are known to occur in many parts of Nigeria, and in certain districts the disease appears to be endemic. The writer has seen cases at Baro and Kateri in Northern Nigeria, but although trypanosomes were present in the peripheral blood of these patients, they did not appear to be ill. In Kabba province of Northern Nigeria it is said to be endemic, and along the course of the Garara river, where the writer had an opportunity of investigating the subject in 1910, it was well known, but apparently uncommon. Sleeping sickness does not seem to be of a virulent type in Nigeria, and it does not at present occur in epidemic form. To account for this fact it has been supposed that the disease must have existed for a great number of years, and that the natives must have acquired a relative immunity. Some such

explanation is necessary since sporadic cases have been identified in so many places, and the local conditions over so much of the country are so favourable for the spread of the disease.

Sleeping sickness as it occurs at Eket, however, presents some special features. It was thought, therefore, that a study of the trypanosome producing the disease might be of interest. For this purpose Dr. Foran, the medical officer in charge of the sleeping sickness investigations, very kindly sent the writer a guinea-pig which had been inoculated from one of his cases. On examination the trypanosome was found to possess some unusual morphological features which are, I think, of sufficient interest to place on record, in view of the peculiar clinical symptoms with which they are associated.

SLEEPING SICKNESS IN EKET DISTRICT

I am indebted to the official reports of Dr. P. F. Foran for the following account of the disease as it occurs at Eket.

Sleeping sickness has, apparently, been known to the natives of the district for a very long time. It has, indeed, existed beyond the memory of the oldest inhabitants. They state, however, that it has become more common lately, and this they attribute to the fact that the present generation is inclined to ignore certain old native laws that forbade an infected person to associate with his fellows. Two phases of the disease are recognised. The early phase, characterised by enlargement of the glands of the neck, is known as *nsipiton;* and the later phase, in which lethargy appears, is called *odongo-idap.** Cases in the former stage are common, but those that have advanced to the latter stage are comparatively rare. The disease appears to be mild, although a number of deaths are reported to occur annually. In September, 1912, Dr. Foran wrote, 'I have only heard of four deaths from sleeping sickness, yet

* The writer, in 1910, found that a similar distinction was observed by the natives of Northern Nigeria. The stage of glandular enlargement they termed *chiwan wiya* (neck sickness), and the stage of lethargy *chiwan berichi* (sleep sickness). Sleeping Sickness Bureau Bulletin, No. 27, p. 236.

nsipiton [the early, glandular, phase of the disease] is very common.' The duration is a matter of years. In the cases collected, symptoms had been present for from one to five years. The great majority of the patients are children or young adults, their ages ranging from about 6 to 18 years. The sexes are about equally affected.

The symptoms are as follows: As a rule the general health is good, there is no marked anaemia, and wasting is present in only a few cases. Irregular fever occurs, especially in the afternoon and evening, and is accompanied by headache and malaise, and terminates in sweating. The tongue is furred. The spleen is almost always enlarged. The expression of some patients is dull and vacant; and there may be oedema or drooping of the eyelids. Muscular tremors occasionally occur. Weakness or lassitude is always more or less well marked. Skin rashes—urticaria and erythema—are present in many cases; but they are also common in uninfected natives. Impotence and amenorrhoea are said to accompany the disease. Enlargement of the cervical and axillary glands is the most constant sign. The glands are freely movable, soft, and elastic; and on microscopical examination are found to contain trypanosomes. Enlarged glands in other sites, such as the groin, do not contain the parasites. On excision, the glands are found to be pale pink in colour. They do not contain pus, and it is noteworthy that those showing signs of induration are found to be free from trypanosomes. The natives believe that the disease is curable by the excision of the glands, an operation which their 'doctors' readily perform. Numerous healthy individuals bearing scars on their necks are to be met with who have, it is affirmed, been cured in this manner.* According to the natives the glandular phase of the disease (*nsipiton*) lasts for about four years before lethargic symptoms develop. From the fact that somnolence is not a common symptom, most of the patients appearing to be in the early stages, it is possible that many of them recover before this stage is reached.

Trypanosomes are found in the gland juice of practically every

* Compare the note on the excision of cervical glands by a native doctor in Kabba province, Northern Nigeria. Dr. W. Morrison. British Medical Journal, June 8th, 1912.

case, but, up to the present, they have not been detected in the peripheral blood. In a few cases presenting the appearances of advanced sleeping sickness trypanosomes have not been found at all. In them the glands are but little enlarged, and, as noted above, indurated glands are generally free from parasites.

The country around Eket is an undulating plateau covered with dense bush, and intersected by numerous waterways. It includes a good deal of swampy land. The district is densely populated. The towns consist of scattered compounds standing some distance apart, and closely surrounded by farms and banana trees. They are usually very dirty, and some cattle, and a good many pigs, goats, and fowls are kept in them. The towns are generally some distance away from the water-side. Their water supply is obtained at two or three spots, which are closely surrounded by bush and trees, and approached along a narrow shaded path. The children and young adults are the water-carriers, and Dr. Foran considers that this accounts for the majority of the cases of sleeping sickness occurring in young people. Tsetse flies are prevalent all over the district. Dr. J. J. Simpson records *G. palpalis* and *G. caliginea* from Eket in his map of Southern Nigeria, showing the distribution of the genus *Glossina;** but, according to Dr. Foran, *G. tachinoides* is also a common species, at any rate, during the months of September and October.† He has observed that the tsetse flies 'appear to follow pigs about more than any other animal, and it is generally easier to catch the flies where these animals are than at the water.'

THE STRAIN OF TRYPANSOME

The trypanosomes used in this investigation were found in the blood of a guinea-pig kindly sent to the writer from Eket by Dr. P. F. Foran. The guinea-pig had been inoculated with cerebro-spinal fluid from a case of sleeping sickness on March 5th, 1913, but the incubation period is not known. The animal reached Lagos on May 8th, 1913, and was found to be infected with

* Bulletin of Entomological Research, Vol. III, part 2, 1912.

† If true, this is a remarkable fact as *G. tachinoides* does not occur so near to the coast elsewhere in Southern Nigeria. It is generally only met with inland, beyond the forest zone.

trypanosomes. It appeared to be well, and, at the time of writing (June 11th, 1913), is still in good health, although it is now 97 days since the date of inoculation. The trypanosomes have never been very numerous in the blood. Their numbers have not sensibly increased during the last month. It is, therefore, impossible to tell to what stage the disease has at present advanced.

The red blood corpuscles of the host exhibit well marked polychromasia and basophilia. In many of them, too, irregularly shaped fragments are to be seen, which stain red with Gimesa's solution.

MORPHOLOGY OF THE TRYPANOSOME FROM EKET

In this paper the flagellar end of the trypanosome will be termed anterior, and the non-flagellar end posterior.

A—*Living, unstained.*

The trypanosome from Eket in the fresh condition, as seen in a drop of blood from an infected guinea-pig, appears as an elongated tapering body of almost homogeneous consistency. The micronucleus can sometimes be distinguished as a small refractile body. The movements are active. Some individuals, which appear to be stouter than the others, are relatively sluggish, and do not move actively about the field. Others, which are long and slender, vibrate exceedingly actively, and also move rapidly across the field of the microscope. The translatory movement seems to be spasmodic, the trypanosome suddenly gliding across the field after having been more or less stationary, but vibrating vigorously all the time, for some moments previously. As a rule, the flagellar end moves forwards in the translatory movements. The non-flagellar end appears to be blunt, the flagellar attenuated. The undulating membrane is conspicuous.

In blood that has been shed for some little time changes may be observed taking place in the form of some of the trypanosomes. The writer has watched these changes on several occasions. They always occurred in long slender parasites, and were accompanied by a diminution of the activity of the trypanosome. The non-flagellar end of the trypanosome appears to round off, and eventually the

body becomes almost spherical. These atypical parasites are occasionally met with in stained blood films. One is illustrated (Plate XXXIII, fig. 22).

B—*Fixed and stained.*

Method of fixation and staining.—The blood films were fixed either with absolute alcohol or with osmic acid. The forms of the trypanosome were found to be the same in either case, but with osmic acid fixation the appearance of the cytoplasm was different if the films were stained very deeply. All the films were stained with Giemsa's solution (about 30-35 drops in 10 c.c. of water); those fixed with alcohol being stained for one hour, and those fixed with osmic acid for from ten to fifteen minutes.

The longest trypanosome measured, up to the present, is 32μ, and the shortest 8μ.

Breadth.—The breadth of the trypanosomes at the widest part varies from 1μ to 2·5μ.

Shape.—The trypanosome when stained is seen to be polymorphic. Long slender forms, short stumpy forms, and intermediate forms are always present; but their relative proportions vary considerably from day to day. The trypanosomes have never been very numerous, and, as a rule, the long slender forms have predominated. The long slender forms are of two types. In the one type the blepharoplast is terminal, and the nucleus elongated (Plate XXXIII, fig. 1). In some specimens the nucleus appears to have a clear area in the middle which gives it the appearance of being made up of two pieces, and the body anterior to it is often attenuated. In the other type the blepharoplast is situated about 1μ from the posterior extremity, which is prolonged beyond it into a blunt snout (Plate XXXIII, figs. 2, 3). The intermediate forms are very variable in size and general appearance. Some are pointed at both ends, others have a blunt posterior extremity, which is occasionally rounded off like the head of a tadpole. The blepharoplast is either terminal or sub-terminal. The body ends anteriorly in a very short 'free' flagellum. The stumpy forms are exceedingly short in some instances (Plate XXXIII, figs. 11, 12), and may measure as little as 8μ. They are relatively very broad,

See page 357

measuring about 2μ. At the anterior end there is a very short 'free' flagellum. The membrane is not broad. The posterior end is quite blunt, and the beginning of the flagellum may form the posterior border of the parasite. The blepharoplast, as a rule, lies at the edge of the trypanosome a little way in front of the posterior end of the body, and on the opposite edge the folds of the membrane pass forwards. The nucleus is large, and is sometimes placed at the extreme anterior end (Plate XXXIII, fig. 13).

From time to time, in films prepared in the usual way, there appear in the blood trypanosomes which have the flagellum free in its entire length (Plate XXXIII, figs. 14, 15). They have not been observed in unstained blood-films, and, indeed, they would be very difficult to make out in fresh preparations. It is, therefore, possible that they may be artefacts; but it is curious that they have never been seen before by the writer in any other animal, although he invariably prepares his blood films in exactly the same way. These forms are long slender trypanosomes with the blepharoplast situated some way from the extremity. The anterior end is drawn out into a filamentous termination. The body measures about 22μ, and the flagellum 31μ.

Contents of the Cell.—In well stained specimens the protoplasm is homogeneous in structure, neither granules nor vacuoles being present. In films that have been over-stained, especially if they have been fixed with osmic acid, both granules and vacuoles appear (Plate XXXIII, figs. 20, 21). The granules are coarse, and occur on both sides of the nucleus. Sometimes they almost fill up the body of the trypanosome.

The nucleus.—The nucleus is oval, and about 2·5μ in length. The nucleus of the long slender trypanosomes with a terminal blepharoplast has already been referred to. In the stumpy forms the nucleus is rounded, and is generally surrounded on all sides by a zone of protoplasm. The nucleus is situated near the centre of the body in the majority of the trypanosomes, but in a few, and these are always stumpy forms, it is placed anteriorly—that is, at the flagellar end (Plate XXXIII, fig. 13). This is an interesting feature, as it suggests that the position of the nucleus in the stumpy forms is an index of the virulence of the trypanosome. In the typical *T. gambiense* it is central, in the more virulent

T. rhodesiense it is sometimes posterior, and in this less virulent strain of Nigeria it is anterior.

Blepharoplast.—The position of the blepharoplast has already been mentioned. It is well marked, round, and deeply staining.

Undulating membrane.—In the long slender forms the undulating membrane is ample, and is thrown into a number of folds. In some it is apparently absent, and the flagellum is, in consequence, free in its whole length. The membrane is less folded in the stumpy and intermediate forms. In carefully prepared specimens the membrane can be followed almost to the extremity of the flagellum, even in the slender forms which at first sight appear to have a long 'free' flagellum (Plate XXXIII, fig. 4).

Flagellum.—The flagellum stains deeply. It is well marked in all forms, and in all forms the terminal portion is free—that is, projects beyond the protoplasm of the cell and the undulating membrane. As already stated, in some forms of long slender parasites, in stained preparations, the flagellum appears to be free in its entire length.

Curve of Measurements:—

In their paper entitled 'Further measurements of *Trypanosoma rhodesiense* and *T. gambiense*'[1] Prof. Stephens and Dr. Fantham have pointed out that in measuring trypanosomes it is advisable to confine observations to a single animal, as it is possible that the size of the parasite may vary in different hosts. They also recommend that the trypanosomes should be measured on ten consecutive days so as to obviate the errors due to the daily variation in the numbers of short and long forms. They suggest that 100 trypanosomes should be measured on each of the first ten days of the infection in a white rat. It has been impossible for me to follow this plan exactly because no white rats have been available, and, in the few animals that have been infected by sub-inoculation, the parasites have been extremely rare at the commencement of the disease, and have, indeed, been apparently absent altogether from the peripheral blood for several days at a time. I have, therefore, measured 100 trypanosomes on each of the ten days from the 96th day to the 105th day after inoculation in the original guinea-pig sent to me from Eket.

With this exception, I have endeavoured to follow the procedure of Stephens and Fantham as closely as possible, so that my figures might be comparable with those given by these authors for *T. rhodesiense* and *T. gambiense*.

Thin blood smears, dried in the air, were fixed in absolute alcohol and stained with Giemsa's solution. The trypanosomes were then drawn with a camera lucida, and the length of a line drawn through the middle of each was measured by the 'tangent line' method. This method is, I believe, the most accurate that has as yet been described.

In the following tables the results of these measurements are given in detail, so that a closer analysis than mere averages would permit may be made by anyone who should wish to do so. A summary of the measurements is added, and a table showing the distribution into the stumpy, intermediate, and long form groups of Bruce.

Table 3 shows the great variation in the number of intermediate and long forms of trypanosome on particular days. The variation is not, however, so great in the percentage of stumpy forms, ranging only between 66 % and 36 %. On comparing this table with that given by Stephens and Fantham, it will be seen that there is a lesser degree of variation in the figures for all three groups, and in particular the number of the intermediate forms is strikingly less. Intermediate forms, that is trypanosomes measuring from 22μ to 24μ, were indeed comparatively scarce, although actually they were among the most common lengths of parasite. The relatively slight degree of variation may have been due to the period to which the disease had advanced in this animal. The figures given by Stephens and Fantham referred to a rat during the first ten days of the disease, those dealt with in this paper were from a guinea-pig between the 96th and the 105th day after inoculation.

TABLE I.—Distribution in respect to length of 1,000 non-dividing individuals of trypanosome from a single guinea-pig

Day after inoculation		In Microns																											Averages	
		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	Of each 20	Of each 100
96th day.	1	...	...	...	1	...	2	...	2	5	...	1	...	2	...	1	1	2	1	1	...	...	...	1	...	...	...	...	18·95	
	2	...	...	...	...	1	...	...	1	4	1	1	1	...	5	1	1	...	2	...	...	1	...	1	...	...	...	...	20·15	
	3	...	...	...	...	...	1	...	1	2	2	2	2	...	2	2	2	2	2	...	...	...	...	...	...	...	...	...	19·90	19·28
	4	...	...	1	...	1	...	2	2	1	1	1	2	1	2	1	1	2	...	...	...	...	...	...	...	...	...	...	18·40	
	5	...	...	1	...	1	1	2	1	1	...	...	2	2	2	2	1	2	1	1	...	...	...	...	...	...	...	...	19·00	
97th day.	6	...	...	...	...	...	...	1	...	...	...	...	1	2	2	4	2	2	2	2	1	...	1	...	...	...	...	...	22·75	
	7	...	...	...	...	...	...	1	...	1	1	2	1	...	2	3	2	1	...	2	1	2	1	...	...	...	...	...	22·20	
	8	...	...	...	...	1	...	2	...	...	1	2	1	1	1	2	3	1	1	1	1	...	2	...	...	...	...	...	21·25	22·20
	9	...	...	...	...	...	...	...	2	...	1	...	...	1	3	5	2	...	2	2	...	...	1	...	...	...	...	1	22·55	
	10	...	...	...	...	...	...	...	1	...	1	1	2	...	1	2	4	2	5	...	1	...	...	...	...	...	...	...	22·25	
98th day.	11	...	...	...	...	...	...	...	2	1	...	1	1	2	1	2	...	2	3	2	2	...	...	1	...	...	...	...	22·35	
	12	...	...	...	...	...	...	...	1	1	...	4	2	...	...	1	4	3	1	...	...	1	2	...	...	...	...	...	21·90	
	13	...	...	...	...	1	...	1	2	1	1	1	4	1	3	...	3	1	...	...	...	1	...	...	...	...	...	...	19·35	20·45
	14	1	...	...	1	...	...	...	2	2	4	...	2	2	1	...	...	...	1	1	2	...	...	1	...	...	...	...	19·15	
	15	...	1	...	...	...	1	...	2	1	2	3	1	...	1	2	1	3	...	...	1	1	...	...	...	...	...	...	19·50	
99th day.	16	...	...	...	...	1	1	1	1	...	...	2	...	...	...	...	...	2	2	1	1	3	2	2	1	...	...	...	23·70	
	17	...	...	...	...	1	...	...	...	...	2	1	2	...	...	1	2	1	...	4	...	3	2	...	...	1	...	...	23·60	
	18	...	...	...	1	...	...	...	...	...	2	5	1	1	3	2	...	1	1	...	1	...	...	2	...	...	...	...	20·85	22·99
	19	...	...	...	...	...	...	...	...	3	...	...	1	2	1	1	1	2	3	...	2	...	3	...	1	...	...	...	23·40	
	20	...	...	...	...	...	...	...	...	1	3	...	...	2	1	...	3	2	3	...	2	...	...	...	2	1	...	...	23·40	
100th day.	21	...	...	...	...	1	...	...	...	...	1	2	6	3	2	3	1	...	...	1	...	...	...	...	...	...	...	...	19·80	
	22	...	...	...	...	...	...	...	1	2	3	3	3	1	2	1	1	2	...	1	...	...	...	...	...	...	...	...	19·50	
	23	...	...	1	...	1	1	...	1	...	1	...	1	3	1	2	1	1	1	4	...	...	...	1	...	...	...	...	20·85	20·31
	24	...	...	...	1	...	...	...	1	2	1	2	2	1	1	5	1	...	...	...	2	...	1	...	...	...	...	...	20·30	
	25	...	...	...	...	...	...	2	2	...	...	3	1	2	1	1	...	2	2	1	2	...	...	1	...	...	...	...	21·10	

TABLE I.—*Continued*

Day after inoculation	In Microns																													Averages	
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	Of each 20	Of each 100
101st day. 26	...	...	...	...	...	...	...	...	...	2	2	1	1	3	3	2	3	...	...	2	...	1	...	...	...	...	...	21·95	
27	...	...	...	...	...	...	1	...	...	...	4	3	1	3	2	...	1	2	1	...	...	2	...	...	...	...	...	21·40	
28	...	...	...	...	1	1	1	1	...	2	...	2	3	...	1	...	1	1	3	1	2	...	...	...	...	...	...	20·90	21·14
29	...	...	...	...	1	...	1	1	...	2	2	2	1	...	1	1	3	2	2	1	...	...	...	...	...	...	...	20·75	
30	...	...	...	...	...	1	1	...	1	...	2	2	1	4	2	4	...	...	1	...	...	...	...	1	...	...	...	20·70	
102nd day. 31	...	...	...	...	...	...	...	1	2	2	...	2	...	...	1	1	1	2	3	2	1	1	...	1	...	...	...	22·90	
32	...	...	...	...	...	...	...	2	...	3	...	2	2	2	...	1	2	2	1	1	...	1	...	1	...	...	...	21·75	
33	...	...	...	...	...	...	...	2	3	3	1	...	...	1	...	...	4	2	...	2	...	...	1	1	...	...	...	21·45	22·96
34	...	...	...	...	...	...	...	1	...	1	...	2	2	1	...	...	1	1	4	1	...	1	...	3	2	...	...	24·85	
35	...	...	...	...	...	...	...	...	2	...	1	1	...	2	2	3	...	1	1	1	5	...	...	...	...	...	1	23·85	
103rd day. 36	...	...	...	...	...	...	...	1	3	...	3	1	4	1	...	1	...	2	1	2	...	...	...	1	...	...	...	21·05	
37	...	...	...	1	...	...	...	1	1	1	1	2	2	3	...	1	1	1	3	...	1	...	1	...	...	...	...	21·30	
38	...	...	...	...	...	...	...	...	1	1	2	5	...	4	1	...	...	3	1	...	...	...	1	1	...	...	...	21·60	20·80
39	...	...	...	...	...	...	2	1	1	2	...	1	1	4	...	2	1	...	...	2	1	1	1	...	...	...	...	21·35	
40	...	...	...	1	1	...	...	2	2	2	1	3	2	3	1	...	...	1	...	...	...	...	1	...	...	...	...	18·70	
104th day. 41	...	1	...	...	...	...	...	1	1	2	2	2	2	...	...	2	1	...	2	1	1	1	1	...	...	...	...	21·20	
42	...	...	...	1	...	...	1	...	...	2	1	1	1	1	2	1	...	3	2	2	1	...	...	...	...	1	...	22·30	
43	...	...	...	1	1	...	...	...	2	1	1	1	1	2	...	1	2	...	1	2	...	1	1	...	2	...	...	22·30	22·82
44	...	...	...	...	...	...	...	...	...	1	1	1	1	...	1	1	...	4	1	2	4	2	...	1	...	...	...	25·00	
45	...	...	...	...	...	1	1	1	...	1	1	...	1	4	...	...	1	...	2	2	...	1	1	2	...	...	1	23·30	
105th day. 46	...	...	...	...	...	...	...	1	1	3	1	2	2	...	2	3	...	1	...	3	1	...	...	...	...	...	...	21·25	
47	...	...	...	...	...	...	...	1	...	2	1	3	3	2	1	1	...	1	2	...	1	...	...	...	1	1	...	22·05	
48	...	...	...	...	1	...	...	...	...	1	4	3	1	2	...	2	2	...	1	...	1	1	...	1	...	...	...	21·40	21·91
49	...	...	...	...	...	...	...	...	1	2	2	1	1	4	...	...	1	2	1	...	...	3	2	...	...	...	...	22·80	
50	...	...	...	...	...	...	1	...	1	...	4	3	1	2	...	...	1	1	1	2	...	1	1	...	1	...	...	22·05	
Totals	1	2	3	8	14	10	21	42	51	64	74	84	64	85	65	64	61	67	58	48	31	32	21	17	8	2	3	Average length of 1,000 trypanosomes is 21·486μ	

TABLE II.—Summary of measurements (in microns) of the lengths of 1,000 individuals of the trypanosome from Eket, Southern Nigeria, from a single guinea-pig.

	Maximum	Minimum	Averages of each 100	Averages of each 20	Range of averages of each 20
1	30	11		18·95	
2	30	12		20·15	
96th day. 3	25	13	19·28	19·90	1·75
4	25	10		18·40	
5	26	10		19·00	
6	29	14		22·75	
7	29	14		22·20	
97th day. 8	29	12	22·20	21·25	1·50
9	34	15		22·55	
10	27	15		22·25	
11	30	15		22·35	
12	29	15		21·90	
98th day. 13	28	12	20·45	19·35	3·20
14	30	8		19·15	
15	28	9		19·50	
16	31	12		23·70	
17	32	12		23·60	
99th day. 18	30	11	22·99	20·85	2·85
19	31	16		23·40	
20	32	16		23·40	
21	26	12		19·80	
22	26	15		19·50	
100th day. 23	30	10	20·31	20·85	1·60
24	29	11		20·30	
25	30	14		21·10	

TABLE II.—*Continued*

	Maximum	Minimum	Averages of each 100	Averages of each 20	Range of averages of each 20
26	29	17		21·95	
27	29	14		21·40	
101st day 28	28	12	21·14	20·90	1·25
29	27	12		20·75	
30	31	13		20·70	
31	31	15		22 90	
32	31	15		21.75	
102nd day. 33	31	15	22·96	21·45	3·40
34	32	15		24·85	
35	34	16		23·85	
36	31	15		21·05	
37	30	11		21·30	
103rd day. 38	31	16	20·80	21·60	2·90
39	30	14		21·35	
40	30	11		18·70	
41	30	9		21·20	
42	33	11		22·30	
104th day. 43	32	11	22·82	22·30	3·80
44	31	17		25·00	
45	34	13		23·30	
46	28	15		21·25	
47	33	15		22·05	
105th day. 48	31	12	21·91	21·40	1·55
49	30	16		22·80	
50	32	14		22·05	
				Range=25·00—18·40=6·60	

TABLE III.—The trypanosome from Eket, Southern Nigeria, arranged in the three groups—(*a*) 21μ and under; (*b*) 22-24μ; (*c*) 25μ and over.

Day	96	97	98	99	100	101	102	103	104	105	Totals
Stumpy 8-21μ	66	36	58	39	62	54	41	67	43	57	523
Intermediate 22-24μ	21	35	22	18	21	24	16	8	12	13	190
Long 25-34μ	13	29	20	43	17	22	43	25	45	30	287
	100	100	100	100	100	100	100	100	100	100	1000

TABLE IV.—Comparison of the trypanosome from Eket, Southern Nigeria, and *T. gambiense* (as given by Stephens and Fantham).

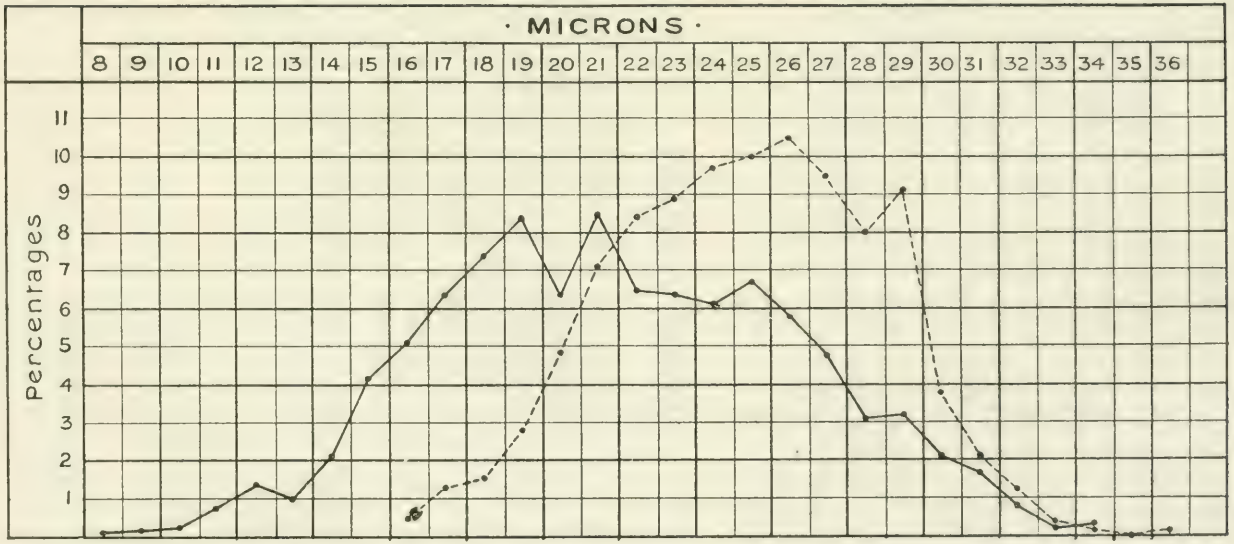
	Average	Maximum	Minimum	Under 22μ	22-24μ	25μ and over
T. gambiense	24·87μ	36·0μ	16·0μ	18·2 %	27·0 %	54·8 %
T. nigeriense	21·486μ	34·0μ	8·0μ	52·3 %	19·0 %	28·7 %

In Chart I a curve is given representing the distribution by percentages in respect to length of 1,000 non-dividing specimens of the trypanosome from Nigeria from a single guinea-pig. The curve given by Stephens and Fantham for *T. gambiense* is added on the chart to facilitate comparison.

The main object of the present investigation was to compare the measurements of the trypanosome from Southern Nigeria with those of a typical strain of *T. gambiense*. The figures have, therefore, been recorded in a manner that will permit of their being compared in detail with those given in what is, I believe, the latest exhaustive study of *T. gambiense*, that is, the paper by Stephens and Fantham already referred to repeatedly.

It will be observed that the trypanosome from Southern Nigeria is somewhat shorter than *T. gambiense;* the average length of

Chart No. I



Graphical representation of the distribution of the lengths of 1,000 trypanosomes from a guinea-pig infected at Eket (continuous line), and 1,000 *T. gambiense* from one rat (dotted line) according to Stephens and Fantham.

1,000 individuals being 21·486μ, as contrasted with 24·867μ. The maximum length of *T. gambiense* as given by Stephens and Fantham is 36·0μ, and the minimum 16·0μ, as compared with 34·0μ and 8·0μ in the case of the Nigerian trypanosome. The curve, too (Chart I), is strikingly different from that given by these authors for *T. gambiense*. It is lower and more extended, and reaches its main peak at 21μ, instead of at 26μ. At the far end, representing the exceptionally long parasites, the two curves almost coincide, but from this point backwards there is no agreement, and when the small forms are reached, the Nigerian trypanosome shows a very much higher percentage than that in the typical strain of *T. gambiense*. It has been already pointed out that the occurrence of these extremely small individuals is characteristic of this strain of human trypanosome.

ANIMAL REACTIONS

Dr. Foran, at Eket, injected a number of rats with blood and gland juice from cases of sleeping sickness, but none of them developed parasites. He used 'three kinds of rats in the experiments, namely, a large black house rat, a smaller brown bush rat, and a brown striped rat'; but 'all appeared to be immune.'

The animal reactions of the trypanosome are at present under investigation. It will, however, be some time before anything definite can be said about them, as the strain seems to be but slightly pathogenic. The following table embodies the results so far obtained:—

Animal	Days since inoculation, up to July 8	Day on which trypanosomes were first detected	Remarks
Guinea-pig 1	124	?	Alive and well. Trypanosomes numerous. The original animal from Eket.
,, 2	56	...	Has never shown trypanosomes. Alive and well.
,, 16	15	...	Died on 15th day. Never showed trypanosomes. Tissues nil.
,, 17	34	...	Has never shown trypanosomes. Alive and well.
,, 22	24	...	Has never shown trypanosomes. Alive and well.
,, 23	24	...	Has never shown trypanosomes. Alive and well.
Mouse 7	28	...	Died on 28th day. Never showed trypanosomes. Tissues nil.
,, 24	16	4	Trypanosomes always very scanty and sometimes absent. Alive and well.
,, 26	10	6	Trypanosomes scanty. Accidentally killed on 10th day.
,, 31	10	8	Trypanosomes very scanty. Alive and well.
,, 32	6	...	Trypanosomes not yet seen. Alive and well.
,, 34	6	...	Trypanosomes not yet seen. Alive and well.
,, 35	6	...	Trypanosomes not yet seen. Alive and well.
Goat 11	34	...	Has never shown trypanosomes. Alive and well.
,, 12	34	...	Has never shown trypanosomes. Alive and well.
Dog 13	34	...	Has never shown trypanosomes. Alive and well.
,, 14	34	...	Has never shown trypanosomes. Alive and well.
Monkey 21	22	10	Trypanosomes always scanty and sometimes absent. Alive and well.

SUMMARY AND CONCLUSIONS

The trypanosome, of which a preliminary account is given in this paper, seems to differ in several respects from a typical strain of *T. gambiense*. In man it produces a form of sleeping sickness that is relatively mild, occurs most commonly in young people, and in which the trypanosomes are, apparently, either absent from the peripheral blood altogether, or present in such small numbers that hitherto they have not been detected. To the smaller laboratory animals the strain seems to be but slightly pathogenic. The morphology of the trypanosome as it appears in the blood of a guinea-pig shows some peculiar features. The trypanosome is smaller than *T. gambiense*, the cell protoplasm when well stained is homogeneous, and there appear constantly in the blood films a few very minute parasites measuring as little as 8μ in length. Some of the short and stumpy parasites have the nucleus situated far forwards at the anterior (flagellar) end of the body. The occurrence of a few peculiar trypanosomes which appear to have a flagellum free in its whole length is also remarkable.

Considering the morphological features of the parasite, and the peculiar symptoms of the disease produced by it, I am convinced that this trypanosome from Nigeria cannot be regarded as belonging to the same species as *T. gambiense*. I therefore propose for it the name *T. nigeriense*.

We regret that it has been found impossible to reproduce Dr. Scott-Macfie's Plate in this number, but it will appear in the next.—[EDS.]

REFERENCE

1. STEPHENS, J. W. W. and FANTHAM, H. B. Further Measurements of *Trypanosoma rhodesiense* and *T. gambiense*. Annals of Tropical Medicine and Parasitology, Vol. VII, No. 1, 1913.

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The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1913:—

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By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is Part B of the third number of the seventh volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

ADDENDUM ET CORRIGENDUM

The following Plate (Pl. XXVIII) is of *Trypanosoma nigeriense*, Macfie, 1913, and illustrates the preceding paper, already published.

In the text of Dr. Macfie's paper the Plate was numbered inadvertently as XXXIII. Hence, on pp. 344-346 the Plate should have been referred to as Pl. XXVIII.

EXPLANATION OF PLATE XXVIII

Trypanosoma nigeriense (Macfie, 1913)

Figs. 1 to 23. Forms of the trypanosome seen in the blood of a guinea-pig. × 2,100.

Figs. 1-5. Long forms.

Figs. 6-9. Intermediate forms.

Figs. 10-13. Short, stumpy forms. In Fig. 13 the nucleus is seen to be near the anterior (flagellar) end.

Figs. 16-19. Dividing forms.

Figs. 20-21. Trypanosomes containing granules and some vacuoles.

Fig. 22. Rounded form.

PRELIMINARY NOTE ON THE DEVELOPMENT OF A HUMAN TRYPANOSOME IN THE GUT OF *STOMOXYS NIGRA*

BY

J. W. SCOTT MACFIE, M.A., M.B., CH.B.

WEST AFRICAN MEDICAL STAFF

(*Received for publication* 14 *September*, 1913)

The rôle of *Stomoxys* in the transmission of trypanosomiasis has been much discussed. The Sleeping Sickness Commission of the Royal Society performed many experiments to determine whether this insect could transmit *T. pecorum*. All the experiments were unsuccessful, and Sir David Bruce and his collaborators concluded that 'It will therefore require very convincing proof to bring this Commission to the belief that *Stomoxys* are carriers of this disease.'[1] A similar conclusion was arrived at by Bevan,[2] in Southern Rhodesia, who failed to infect sheep with the Hartley trypanosome by means of *Stomoxys* flies. On the other hand, Bouet and Roubaud,[3] in French West Africa, succeeded in transmitting *T. cazalboui*, *T. pecaudi*, *T. sudanense*, and *T. evansi* by means of *Stomoxys* (probably *S. calcitrans*). They did not, however, observe any developmental forms in the digestive tract or proboscis of the insect, and although they considered the species of fundamental importance in the etiology of trypanosomiasis, they were not able to regard it as a typical intermediary host. In Northern Nigeria also this genus has fallen under suspicion, and the writer has recorded one case in which an infection with *T. brucei* (*pecaudi*) was probably conveyed to a horse by *Stomoxys nigra* or *Stomoxys calcitrans*.[4]

Last May, the writer had an opportunity of experimenting with flies of this genus at the Medical Research Institute, Lagos. As he succeeded in breeding *Stomoxys nigra* in captivity, it was hoped to investigate thoroughly the rôle of this insect in the transmission of trypanosomiasis. The experiments were however interrupted, and

as it seems unlikely that they can be resumed for some time, the following incomplete records are given by way of a preliminary note.

Towards the end of May, and at the beginning of June, 1913, a number of *Stomoxys nigra* flies, caught in the laboratory, were fed on a guinea-pig infected with the trypanosome from a case of sleeping sickness from Eket in Southern Nigeria. The morphology of this trypanosome, which is being described elsewhere, differed in several respects from that of *T. gambiense*. Thirteen of the flies were dissected from one to six days after the first infecting feed. In six of them flagellates (Herpetomonas) were found in the mid-gut. As a control, twelve flies that had not fed on the infected animal were dissected. No flagellates were found in them.

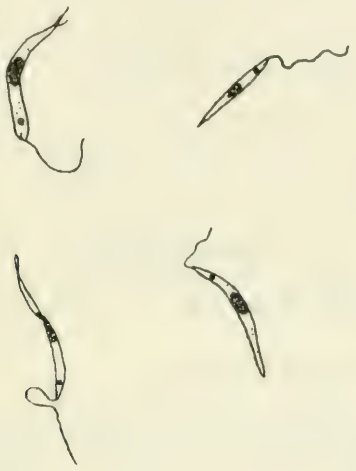
Unfed Flies			Fed Flies		
Number Dissected	With flagellates in the gut	Without flagellates in the gut	Number Dissected	With flagellates in the gut	Without flagellates in the gut
12	0	12	13	6	7

From these observations it was thought probable that the presence of the flagellates in the mid-gut must be due to the infecting feeds on the guinea-pig. As, however, it was possible that the flies might have been naturally infected, experiments were begun with flies hatched out in the laboratory.

Experiment 1. On June 14th a *Stomoxys nigra* fly that had hatched out on the previous day was fed on the infected guinea-pig. On June 17th the fly was killed and dissected. Herpetomonas were found in the mid-gut. No flagellates were present in the salivary glands and proboscis. The attached sketches, drawn with a camera lucida, illustrate some of the flagellates found in the mid-gut of this fly.

Experiment 2. On June 28th a *Stomoxys nigra* fly which had hatched out the previous day was fed on the infected guinea-pig. On July 2nd the fly died, and was dissected. Herpetomonas were found in its mid-gut. No flagellates were seen in the salivary glands and proboscis.

Each fly received only a single infecting feed. Two other specimens of *Stomoxys nigra* that had been bred out in the laboratory, but had not been fed on the infected guinea-pig, were dissected. No flagellates were found in either of them.



Herpetomonas (× 1000) from gut of *Stomoxys nigra*.

At this stage the experiments had to be abandoned. Some apology is necessary for recording such incomplete observations. The two experiments given above were, however, quite definite, and would seem to prove that the trypanosome with which the guinea-pig was infected was capable of development in the gut of *Stomoxys nigra*. I am not aware that this fact has previously been established, and its importance must be my excuse for publishing this note. In Nigeria, in the native towns and European stations, *Stomoxys* flies abound. If, as appears probable, they are capable of serving as the intermediary host of human trypanosomiasis, they deserve greater consideration than they at present obtain.

MEDICAL RESEARCH INSTITUTE,
LAGOS,
SOUTHERN NIGERIA,
August, 1913.

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3. G. Bouet et E. Roubaud. Expériences de Transmission des Trypanosomiases animales de l'Afrique Occidentale française par les Stomoxes. Bull. Soc. Path. Exot., 1912, v. No. 7, pp. 544-550.

4 Ann. Trop. Med. & Parasit., 1913, VII, No. 1, p. 9.

A NOTE ON A CASE OF *LOA LOA**

BY

W. ROGERS, M.D.

(*Received for publication* 30 *August*, 1913)

From May, 1906 to June, 1907, I was in Southern Nigeria; beyond a few slight attacks of fever I was in good health. From July to November, 1907, I was in Wales, and had evanescent thickening of one or other leg, and, generally, an oedema about the ankle, which puzzled me, and which lasted a day or two; various diagnoses were made, including rheumatism, cellulitis, erythema, nodosum, etc.

From November, 1907 to December, 1908, I was in Lagos Protectorate; no symptoms beyond an occasional oedema about the ankle of one or other foot; no fever. December—March, 1909, travelling on Continent. In March, 1909, I settled down in Wales and had no symptoms until the late summer, when I began to notice fugitive swellings in various parts of the body, generally in the neighbourhood of joints; painless, but associated with stiffness. The general diagnosis was rheumatism, although one consultant of note diagnosed Angioneurotic oedema. In November, 1909, I recognised *Filaria loa* crossing the bridge of the nose, since which time there has been no prolonged freedom from signs of the presence of the worm.

As a rule, I became suddenly aware of a stiffness in a certain part of the body; on examination I would find that part swollen, sometimes to a considerable extent. At first the swelling was wont to be localised, but later it would involve the greater part of the circumference of the limb. I once had an upper arm that Hackenschmidt would envy! When the parasite appeared on the face I was generally made aware of its presence by accidentally feeling a spot that was tender on pressure, say, in the lower jaw;

* The following note is of considerable value as representing Dr. Rogers' description of the symptoms in his own case.—J. W. W. Stephens.

later on, in an hour or two, that part was swollen but the tender spot had passed upwards towards the eye, always leaving in its wake an oedematous track. I noticed that invariably when it appeared on the face it made its way towards one or other eye: it generally made one or two tours round the eye, either in the lid or under the ocular conjunctiva, and then crossed the bridge of the nose to the other eye; in the region of the eye it was always visible under the skin. After visiting the eye it would wander over the scalp, its curve being easily traceable by the tender area and, later, localised swelling. The next day possibly, I would feel nothing further, and so for several weeks; or, on the other hand, I might wake up with a swelling in a leg or arm; in this case it is impossible to say whether it was the same worm or no, as it was later shown that I had at least two parasites. The presence of the worm in the eye was always accompanied by a pricking, boring pain; when under the conjunctiva it caused severe conjunctivitis with redness, dilated vessels, feeling of grit, headache, etc.

On one occasion a cutting operation was attempted without success; on another, an injection, likewise without success, of 1/1000 perchloride. After these attempts it disappeared for several weeks. Towards the end of 1912 (about October, I think) a worm was removed from under the conjunctiva, on which you kindly reported, describing it as an immature female. After this there were no more ordinary, usual symptoms for some time. In December, 1912, I developed 'Mumps,' and was accordingly isolated; in the light of later knowledge I have no doubt this was caused by the second parasite. After that I had rest until a month or two ago, when, after the prolonged hot weather, the old signs reappeared, but in an exaggerated form. A swelling would appear in a limb, at first localised (exactly like a lipoma), but later on (a few hours) firm, brawny and, for the first time, *red*, looking like a cellulitis. The limb (arm or leg) would be quite elephantoid: on one memorable occasion the thigh was so swollen that I was unable for several hours to put on my trousers. *On that occasion the parasite, if it was the same one, had been seen in the eye the previous day.* The swelling would be totally gone in a day or two. When it appeared in the eye it would leave that organ swollen and closed, giving me a 'drunk and disorderly' appearance; that, with

the accompanying headache and woebegone look, were likely to give rise to unwarrantable suspicions detrimental to my character.

During the first two or three years I made numerous blood examinations, but found nothing of interest. At first the red cells were poor in shape and colour, and I several times saw the parasites of tropical tertian fever. In 1909-1911 my mononuclear count was about 20 per cent. and I had an eosinophilia of 3-5 per cent., which was not surprising seeing that in addition to this *F. loa* I suffered from Bilharzia (contracted during the South African War), and from which I was not altogether free until the end of 1911. I have made no blood examination since November, 1911.

A second worm, now in your possession, was removed in the middle of August, 1913. (This worm is a mature female. J. W. W. Stephens.)

RECENT EXPERIMENTAL RESEARCH BEARING UPON BLACKWATER FEVER

BY

J. O. WAKELIN BARRATT, M.D., D.Sc. (LOND.)

(*Received for publication* 24 *June*, 1913)

In 1909 Barratt and Yorke* in an investigation into the mechanism of production of blackwater showed that in this condition haemoglobinuria was preceded by, and dependent upon, haemoglobinaemia. It was also shown that suppression of urine, occurring in blackwater fever, was due to mechanical blocking of the uriniferous tubules. In one experiment in which haemoglobinaemia was induced in a rabbit by the injection of haemoglobin, death occurred suddenly at the end of eighty-five minutes; in all similar experiments, however, the general condition of the animals injected was not affected.

As subsequent papers bearing upon these observations are somewhat scattered it will be of advantage to give a brief résumé of the results obtained.

Barratt† (1910), in order to ascertain the extent to which the haemolytic action of quinine salts upon red blood cells was due to hydrolytic dissociation, determined the first and second dissociation constants of this alkaloid.

Barratt and Yorke‡ (1909) extended their observations to piroplasmosis in the dog, and showed that in this condition haemoglobinuria makes its appearance as soon as the blood plasma contains as much haemoglobin as is obtainable from an amount of red cells equal to 0·5 per cent., by volume, of the plasma, the

* J. O. W. BARRATT and W. YORKE. 'An investigation into the Mechanism of Production of Blackwater.' Annals of Trop. Med. & Parasit., 1909, Vol. III, p. 1.

† J. O. W. BARRATT. 'Uber die Konstanten der ersten und zweiten Dissociation des Chinins.' Zeitschr. f. Elektrochemie, 1910, B. 16, S. 130.

‡ J. O. WAKELIN BARRATT and W. YORKE. 'Uber den Mechanismus der Entstehung der Hämoglobinurie bei Infektionen mit *Piroplasma canis*.' Zeitschr. f. Immunitätsforschung u. exp. Thorapie, 1909, B. 4, S. 313.

percentage of haemoglobin in the urine being generally considerably greater than that contained in the blood plasma. Haemoglobinaemia and haemoglobinuria do not make their appearance in this condition until an extensive destruction of red cells has taken place, the latter often showing at the time of death a diminution to one-fifth of the number originally present; the plasma, on the other hand, remains little altered in volume. In two cases jaundice was observed.

Yorke and Nauss* (1911) showed that the injection of a haemoglobin holding fluid in sufficient amount was frequently followed by severe symptoms, rapidly terminating in death. In this connection attention may be directed to the severe symptoms attending blackwater fever during the period of haemoglobinuria; the similarity of the symptoms in the two cases suggests that the mechanism of production is the same in both. Yorke and Nauss also made the important observation that suppression of urine in some cases followed the injection of a solution of haemoglobin. It was found that this result was obtained if a sufficient amount of the haemoglobin was injected in an animal which had previously been deprived of water for some time, the occurrence of a greatly increased secretion of urine, which would otherwise follow upon injection, being thus prevented.

Yorke† (1911) observed that, in haemoglobinaemia due to *Piroplasma canis* or produced by the intravenous injection of haemoglobin, granules reaching as much as 3μ in diameter were found in large numbers in the epithelium of the convoluted tubules of the kidney, indicating that the seat of elimination of haemoglobin is the epithelium of these tubules. Illustrations are given of sections of the kidney after injection.

Barratt and Yorke‡ (1912) in the course of a further investigation showed that the acute symptoms following injection were not attributable to haemoglobin, but to substances derived from the stromata of the red cells. For the production of anuria the

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* W. Yorke and R. W. Nauss. 'The Mechanism of the Production of Suppression of Urine in Blackwater Fever.' Annals of Trop. Med. & Parasit., 1911, Vol. V, p. 287.

† W. Yorke. 'The passage of Hamoglobin through the Kidneys.' Annals of Trop. Med. and Parasit., 1911, Vol. V, p. 401.

‡ J. O. W. Barratt and W. Yorke. 'Ueber Hämoglobinämie.' Zeitschr. f. Immunitätsforschung u. exp. Therapie, 1912, B. 12, S. 333.

introduction of haemoglobin in relatively large amounts into the circulating blood was shown to be necessary, but here also the presence of substances derived from the stromata was found to be a factor in the determination of anuria. The blood generally coagulated less rapidly after the injection of a solution of haemoglobin. The observations made did not, however, admit of a conclusion being arrived at as to the exact mode in which the general symptoms following injection were brought about. In a subsequent investigation* by the same observers these conclusions were confirmed.

Barratt† (1913) pointed out that the granules in the convoluted tubules of the kidney, observed in the course of haemoglobinaemia, represent mitochondria, which in this condition become remarkably enlarged, and are very readily stained.‡

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* J. O. W. Barratt and W. Yorke. 'The Production of general symptoms in haemoglobinaemia.'

† J. O. W. Barratt. 'Changes in Chondriosomes occurring in pathological conditions.' Quart. Journ. of Micro. Sci., 1913, Vol. LVIII, p. 553.

‡ In this connection it may be pointed out that Lebedeff ('Zur kenntniss der feineren Veränderungen der Nieren bei Hämoglobinausscheidung.' Virch. Arch., 1883, B. 91, S. 267) and Afanassiew ('Über die pathologisch-anatomisch Veränderungen in den Nieren und in der Leber bei einigen mit Hämoglobinurie oder Ikterus verbundenen Vergiftungen.' Virch. Arch., 1884, B. 98, S. 460) both give illustrations of the condition of the kidney in haemoglobinuria, in which the chondriosomes of the convoluted tubules appear to be imperfectly represented. Marchand ('Uber die Intoxication durch chlorsaure Salze.' Virch. Arch., 1879, B. 77, S. 455) regarded the elimination of red blood cells and portions of red blood cells as taking place through a kind of diapedesis; the structures in question being evidently hypertrophied mitochondria, which form striking objects in sections of the kidney during marked haemoglobinaemia.

A FICTITIOUS NATIVE DISEASE *(ISIGWEBEDHLA)*

BY
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(*Received for publication*, 3 *September*, 1913)

For many years reports have been received from natives and from Europeans resident in Zululand that a serious and often fatal disease, known as Isigwebedhla, appeared regularly in epidemic form about the same time as malaria. The outstanding feature of this complaint was stated to be rapid ulceration of the rectum and anus, described by some as gangrenous rectitis. The disease was said to be characterised by sudden onset and fever. Within three or four days the anus 'ulcerated,' in many cases to such an extent that the closed fist might pass through it with ease, after which the ulceration went on to attack the genitals, and even the mouth and throat. As a rule there was a copious passage of green bile; sometimes, however, mucus tinged with blood was passed, or even grey sloughs. In very severe cases the disease 'attacked the back of the neck.' Blood might appear in the urine, and in rare cases there was haemorrhage from mouth and nose. The condition rarely persisted for any time. In the majority of cases the patient either died, or the disease, even when associated with the most extensive ulceration, rapidly cleared up. This account of the condition was corroborated by practically everyone I interviewed on the subject in Zululand. It must be added, however, that few Europeans had actually seen a case, and their impressions of it were derived from native accounts. Only one post-mortem examination on a fatal case had been made, and Dr. Manning, who performed it, said that he could find no ulceration of the rectum whatever.

On arrival in Zululand I went somewhat further into the local evidence, and summarise the more important particulars below.

(1) Isigwebedhla made its appearance shortly after the arrival of the British in 1823, an event of political significance only.

(2) It occurs annually in epidemic form during and after the rains.

(3) There is a great variation in the severity of the annual epidemic, and in some years it is scarcely noticeable.

(4) This epidemic is always associated with a co-existent epidemic of malaria ('Umkuhlane').

(5) Isigwebedhla occurs only in the bush veldt among the Zulu people, unlike malaria which is endemic on the coast flat among the Tonga and mixed race found there.

(6) It has no relation to the tsetse belts, or to the parts infested with the tick *Ornithodorus moubata* which carries the parasite of relapsing fever.

(7) Dietetic changes, as the use of green mealies, new mabele (millet grain), etc., have no influence on its appearance. The new crop of cereals begins to be used at the time of its greatest incidence.

(8) One attack does not protect against a second, and a person may suffer two, or even more, times from it in the course of a single year.

Accompanied by a posse of native doctors, I watched the progress of nineteen cases, each and all of which were diagnosed by these persons as typical and severe 'Isigwebedhlas.' In eighteen of these I demonstrated either in the peripheral, splenic, or hepatic blood the presence of *Haemamoeba praecox* of malignant tertian malaria. In some instances it was in prodigious numbers. All my cases showed the other cardinal signs of malaria, e.g., pigmented leucocytes and a mononuclear increase, and all recovered rapidly under quinine injections. The clinical types noted were as under:

14 were typical bilious remittents.

2 were of the dangerous algid type with great cardiac weakness and a tendency to collapse.

3 were cerebral in type, 2 showed coma, and the other was characterised by convulsions, delirium and diarrhoea.

True dysentery was not a feature of any of them, although in one case the motions contained blood, but only for a day.

In all, the malarial intoxication was sufficient to markedly lower the tonicity of the sphincter ani, and permit of its easy dilation.

In two cases stated to be exceptionally severe, slight rectal prolapse was present. Both patients were more or less collapsed, and in one the condition was undoubtedly aggravated by a retroverted uterus and two days of irritating diarrhoea.

In all, the rectal mucosa participated in changes similar to those observed in the buccal mucosa in any case of fever. Trifling excoriation was observed in most, and was invariably described by those present as ulceration. No organisms were ever found in the tissues below the 'ulcerations,' and scrapings taken from the parts the natives considered ulcerated showed only the ordinary organisms of the intestinal tract. In the case noted above, in which convulsions were a feature, the diarrhoea was apparently due to a small spirochaete, identical as to staining reactions, and presenting the same relations with the endothelial cells of the gut, as that described by Le Dantec. Certain 'abdominal' features of the case may have been due to this organism, but I do not consider that its importance in this instance as a disease-producing factor compares with the rôle played by the sub-tertian malaria parasite, with which even the peripheral blood was heavily infected. This spirochaete is said to produce a mild diarrhoea, chronic in type, in the South of France, and it is possible it may do the same in Africa. It can be easily overlooked on account of its small size and the difficulty of staining it. Those found by me measure 5 to 10μ by 0·5μ in width. They stain faintly pink by Leishman, and are best shown by weak carbol-fuchsin. They do not take Gram. They are seen as minute coiled threads little longer than a red blood corpuscle. They may occur almost in pure culture, forming in places dense felted networks. A few are seen clinging to the free surfaces of the endothelial cells. They were absent from scrapings taken from the rectal mucosa, and must have had their origin higher up. None of my cases showed *Sp. vincenti*, nor organisms which could be identified with the *Bacillus fusiformis*.

Herpes on the lips, buttocks and perineum was occasionally observed. This is a common concomitant of malaria, and explains the reports of 'ulceration involving the skin.'

In the mouth sores, and those involving the throat, in the only case in which the latter was ulcerated, nothing of interest was seen.

Sp. dentium was once demonstrated, and in two instances I found extensive infection with *Sp. buccalis*.

The absence of eosinophilia in all precluded the likelihood of serious hook worm infection. I searched the dejecta in each case, and failed to find eggs or embryos. Ascarides and tapeworm were common enough. I saw no cases of rectal bilharzia. Routine examination of hepatic blood failed to disclose Leishman-Donovan bodies, and none of these cases showed trypanosomes or *Sp. duttoni*, a matter of some interest when compared to the state of affairs in the low veldt proper, where the existence of Isigwebedhla is denied.

It becomes necessary, however, to explain the apparent discrepancy between native accounts of the disease and the actual facts as determined by my investigation.

The initial treatment of most acute diseases, and specially of specific fevers among the Zulu, consist in taking an enemetic followed by an enema. This is done to remove 'bile.'

Emesis is frequently produced by drinking plain water, but in the majority of instances a host of bulbs, roots and barks pounded up, and used either singly or in combination, are infused in hot water which is then drunk. Most of these substances are harmless, many of them, as the Mfusamvu (*Pittosporum viridiflorum*), act in virtue of their soapy nature, a few are true emetics, and some, as the Macapazane (*Bowiea volubilis*), the juice of which sets up a papular rash on the skin and proves toxic to guinea-pigs in small doses, are irritants.

The rectum is emptied as follows: The patient is placed in the knee-elbow position with the chest resting on the ground. A cow-horn with the point cut off is inserted into the anus and a watery infusion of an extensive series of roots, bulbs, etc., is poured in until the bowel will hold no more, when the enema is evacuated. This process is repeated until the fluid comes away clear, and is continued daily. The enema is often followed by a suppository, or the parts are painted with a decoction of various bulbs. I have been told that in Isigwebedhla a suppository of mud and red chillies is sometimes used, 'with excellent results.'

After one or more days of severe malarial fever the tone of the sphincter ani is so reduced that traction on the buttock of a patient in the knee-elbow position results in an immediate dilatation of the

anus. The degree of patency depends on the success with which one can overcome the resistance of the sphincter, and, other things being equal, varies directly with the gravity of the constitutional effects of the fever. The patency, which in many of my cases was extreme, is to the native mind the 'eating away of the flesh,' i.e., ulceration, and is the one cardinal feature of the 'disease.'

It is customary in these cases to keep the patient sitting up throughout the course of the sickness, to facilitate the easy flow of bile. This is done by relays of friends, and it is not surprising to find the unfortunate patient unable to hold up his head after two or three days. On complaining of pain in the spine or back of the neck, the diagnosis that 'Isigwebedhla' has affected these parts is quickly arrived at.

Malaria is, if anything, more prevalent among the coast people than among the Zulu of the hills. There are places in Hlabisa district where one sees cases of 'Isigwebedhla' in the Zulu kraals among the hills, and hears of dozens in the immediate neighbourhood. Not two miles away on the flat are people who are suffering from malaria in as great a degree, yet deny the existence of 'Isigwebedhla' among themselves, and invariably affirm that it is a condition confined to the Zulu.

I consider that the term 'Isigwebedhla' is an invention of some astute practitioner in the past, and have had some strong hints as to the accuracy of this view of it given me by Tonga 'doctors' whose clientele embraces Zulus. Rightly or wrongly, the Tonga doctor has a greater reputation than his Zulu confrère, and it is not only possible, but likely, that this myth was evolved by the Tonga.

There is no doubt that in judging of the severity of such a case the native is swayed more by his deductions from the constitutional symptoms than by the presence of definite lesions in the rectal mucosa, and he is much influenced by the rapidity with which the constitutional symptoms develop. At the same time he is unable to imagine grave constitutional symptoms existing independently of a gross lesion to account for their production. Given delirium or coma (unless he can explain it by witchcraft) he readily constructs an alarming pathology to account for its existence. A comatose case is 'dead' of Isigwebedhla; he may take a hasty glance at the rectum. If no 'ulceration' is seen, or the patency is not marked,

the former must certainly exist higher up. He would never ascribe symptoms to a blood condition *per se*, even if he could conquer his intuition to ascribe such serious disease to witchcraft, an idea which is strengthened by the absence of gross morbid changes.

Seeing, therefore, that 'Isigwebedhla' is merely an advanced stage of an acute febrile disorder, it is difficult to refrain from admiring the acumen by which these ignorant (sic) practitioners, recognising their utter inability to effect a cure, have evolved this terrible and fatal malady, the successful treatment of which is unexpected at best, and which, if achieved, cannot fail to reflect the highest credit on all concerned in it.

ON 'VOMITING SICKNESS' IN JAMAICA

(Report of the XXXth Expedition of the Liverpool School of Tropical Medicine)

BY
HARALD SEIDELIN, M.D.

(Received for publication 25 June, 1913)

PLATES XXIX-XXXIII

In December, 1912, the Committee of the Liverpool School of Tropical Medicine resolved to send me to Jamaica in order to investigate the nature of the disease called 'vomiting sickness,' prevalent in that island during the winter months, and responsible for a considerable mortality, chiefly amongst native children. It was considered of particular importance, for obvious reasons, to determine whether a relationship exists between 'vomiting sickness' and yellow fever.

The Committee of the School received, through the Colonial Office, the assurance that the Government of Jamaica would do all in its power to further the objects of the expedition.

I left Southampton on December 18th, on the R.M.S.P. 'Thames,' and arrived in Kingston, Jamaica, on January 10th, 1913. The Government, through the Superintending Medical Officer, Dr. J. Errington Ker, at once made suitable arrangements in order to facilitate my task. It was agreed that I should establish my headquarters in Kingston, where I could carry on laboratory investigations, and at the same time be in easy communication with all parts of the island, always being ready to go out into the country at a moment's notice. Instructions were issued to all the District Medical Officers, requesting them to report by telegraph all cases of 'vomiting sickness' which might come to their notice, and to make arrangements for post-mortem examination if the cases ended fatally before my arrival. These instructions were faithfully carried out, and on January 13th I performed my first post-mortem examination at May Pen. The only drawback, which was some-

times seriously felt, was that of the very considerable distances which I had to travel in order to reach a case. Cases often occurred out in the bush; in many of these cases the authorities took the trouble of transporting the patient or body to the nearest hospital or mortuary; but this was not always possible, and sometimes it was necessary to travel first several hours by rail, and then several hours in a buggy, besides walking the last part of the way. Two inconveniences resulted: that it was impossible to see even approximately all the cases occurring and reported; and, secondly, that I usually arrived late, in fact, as a rule, after the patient had died, and in some cases even somewhat late for a post-mortem examination. The transport of material to Kingston, for pathological and bacteriological examination, was also sometimes delayed more than was desirable.

Under these conditions I was unable to see more than about half the number of cases reported during my stay in Kingston. One part of the island, the parish of Trelawny, I was particularly anxious to visit, as the majority of the cases diagnosed by Captain Potter as yellow fever had been observed there. I had, however, no opportunity of doing so; very few cases occurred this year in Trelawny, and these I could, for various reasons, not reach in time. Otherwise I saw cases, sometimes alive, but more often after death only, in nearly all the various localities where the disease occurred, and my material includes, as far as I can judge, all the divers types which, with any degree of reasonableness, can be included in discussions on 'vomiting sickness.' Altogether, I saw sixty-two cases (besides several which could at once be otherwise diagnosed), a fairly large number, considering that my whole stay in Jamaica lasted only ten weeks. In the meantime I carried on laboratory work in the Laboratory of the Public Hospital, Kingston; here I had the invaluable assistance of the Government Bacteriologist, Dr. H. H. Scott, who received the material which I sent in from the country, incubated and examined many of the cultures, and always helped me in every possible way. Without his kind assistance I should have been obliged either to refuse a large number of calls or to leave the larger part of the laboratory work undone.

The described conditions of work in Jamaica made it impossible to investigate all epidemiological, clinical, anatomical and micro-

biological details in each case as fully as might have been desirable. The histological work was only just begun in Jamaica, but was almost wholly carried out in Liverpool, and so far under better conditions, as no fresh cases of 'vomiting sickness' were continually claiming the attention of the worker. But various reasons made it desirable not to devote too much time to this part of the investigation. Other work was waiting at home and it was desired that I should as soon as possible proceed to Africa on important investigations. Under the circumstances the necessary examination of sections was done to establish an anatomical diagnosis in doubtful cases and confirm it in others, and illustrations were prepared in order to show the more important histological changes, but no attempt was made to describe the microscopical appearances in all details. This would have been a work of years, whilst at present only about two months could be devoted to it. Instead of delaying the publication of the report it was therefore thought advisable to publish it as soon as possible, especially because there seemed to be no probability that a closer study of histological details, though in itself extremely interesting, would throw any further light on the one question of paramount importance, the question of etiology. I must, however, reserve for myself the right of publishing later on more detailed descriptions of some of the cases which present certain features of more than ordinary interest, though, I believe, without any bearing upon the question of the nature of 'vomiting sickness.'

On March 22nd I sailed from Kingston on the R.M.S.P. 'Oruba,' and on April 15th I arrived at Southampton.

The report is divided into the following sections:—

I. Historical notes (p. 380).
II. Recent observations (p. 386).
III. Personal observations (p. 390).
IV. Discussion of personal observations (p. 393).
V. Description of vomiting sickness (p. 450).
VI. General discussion of its nature (p. 454).
VII. Prophylactic measures (p. 466).
VIII. Other observations in Jamaica (p. 466).
IX. Other places visited (p. 467).
X. Acknowledgments (p. 468).

I. HISTORICAL NOTES

According to Potter (1912) the term 'vomiting sickness' has apparently been in use in Jamaica for many years. My own impression was the same, but I could obtain no definite information. Being a disease which chiefly attacks native infants and children, it seems quite likely that it has not attracted the attention of medical men to any considerable degree, as infantile mortality has been and still is very high, especially among the natives. Native children are often ill, and die, without medical attendance, and in cases of 'vomiting sickness' it must be admitted that parents have one particular and often very good reason for not applying for medical aid, namely the extreme acuteness of the cases which, in combination with the long distances in many cases, makes it useless to send for a doctor as the child is usually dead before he arrives.

In the medical literature 'vomiting sickness' is apparently mentioned for the first time by Turton (1904), who gives a general description of the disease, summing up in the following way:—

'We have here a disease which occurs only in the cold months, in country districts, among the poorer classes and unhealthy surroundings; may attack more than one member of the family, either at the same time or at intervals of hours up to a day or two; usually attacking children irrespective of sex; presenting a longer or shorter period of *malaise*, followed by vomiting, convulsions, and death in a few hours; showing, *post mortem*, signs of gastro-intestinal irritation, and usually infection, with ascarides in large numbers; killing by respiratory failure; and having a very small death rate in cases where early treatment has been given.'

Turton is of opinion that the post-mortem appearances are due to 'venous congestion,' and has, in fact, constantly found hyperaemia of the following organs: cerebral and spinal meninges, lungs, liver, spleen, kidneys, and stomach. Some importance is apparently given to the presence of Ascarides, which were found in nearly every case in the small intestines, sometimes in enormous numbers and forming knots, causing distension of the bowel and irritation of its wall.

In earlier years the same disease may have figured in the Health Reports under other headings, especially under that of cerebro-

spinal meningitis; this question is discussed by Potter (1912, Appendix A). In 1903 the term 'vomiting sickness' appears as a synonym of gastro-enteritis (Annual Report of the Superintending Medical Officer), and in the following years the disease is referred to as 'vomiting sickness.'

In 1905 the Superintending Medical Officer, Dr. J. E. Ker, draws attention to the prevalence of 'vomiting sickness' and to the uncertainty which reigns with regard to its nature; the great interest and importance of the matter are pointed out, and the following points are stated as certain:—

'1. The disease appears at a certain fixed time of the year, November to March, at a time when the temperature varies greatly from day to night.

'2. It rarely appears in town; none of the cases reported have come from towns.

'3. The people attacked are chiefly, but not always, children.

'4. It appears so suddenly and runs its course so quickly that medical men never hear of two-thirds of the cases until after death has occurred.

'5. Frequency with which several members of a family are attacked.'

Ker states that the affection has been returned under various names on the death certificates, and quotes 'Cerebro Spinal Meningitis, Gastritis, Cerebro Spinal Fever, Vomiting and Fits, Black Vomit, Vomiting and Pain, etc.'

After this introduction Ker publishes a number of reports on 'vomiting sickness' by several District Medical Officers who were in possession of considerable experience with such cases.

Dr. Cooke, Mandeville, writes, 'There is no such thing as "Vomiting sickness." The vomiting is simply a symptom as it is of many other diseases. The disease is acute congestion of the lungs, occurring in children under 5 years of age, and only in the poor, ill-fed, and almost naked.' The description of cases given by Cooke appears to bear out his contention.

Dr. Thomson, Chapelton, describes an outbreak of 'vomiting sickness which occurred after a period of unusually cold weather. 'Cases occurred at the same time in widely separated portions of the district. The disease has been more virulent than in previous

years, more adults have been attacked than in previous epidemics, and the death rate has been enormous. So rapid is the course of the disease, that in many cases there has been no time to obtain assistance. Very few cases of the disease are seen, only those occurring in the neighbourhood of the village are reported, the others being heard of only after death has occurred, being reported as sudden death to the police.' Two typical examples of the history are given as follows:—'1. A young child of healthy appearance is put to bed. The parents are roused during the night by a cry and find the child foaming at the mouth, retching, unconscious and cold, with twitching of the limbs, which passes into actual convulsions. 2. A young adult, more often female, leaves home for market—is attacked on the way with retching, vomiting, and intense weakness, becomes unconscious, and is brought home a corpse. Post-mortem examination of these cases reveals no naked-eye appearances of disease. The mucous membrane of the stomach may or may not be congested from the vomiting. There is never any trace of an ulcer. No irritant poison has ever been detected in the cases submitted for analysis. There are never any signs of disease in the kidneys or meninges.'

Dr. Earle, May Pen, describes the disease as apparently 'a severe form of gastritis, with rapid collapse and shock,' and states that it is 'very fatal, especially amongst children.'

A report by Dr. Bell, dated 1900, deals with the disease as observed in the same district, May Pen. He gives detailed descriptions of both clinical symptoms and post-mortem findings. Malaise with epigastric pain is followed by mucous vomiting, and prostration. After an interval of apparent improvement the vomiting returns and collapse sets in. Death may occur after a more or less rapid course. In some cases children were found during night with convulsions, which soon ended in death, or vomiting set in suddenly, with collapse, and death ensued in a few hours. At the autopsies hyperaemia of meninges, brain and liver was observed; the spleen was enlarged, but only slightly congested; the kidneys were intensely hyperaemic, and the stomach showed ecchymoses in the mucosa. The mortality was only about 2 per cent. if treatment was instituted early; otherwise, treatment was of no avail. The term 'epidemic gastritis' is not considered

satisfactory, and no definite opinion is stated with regard to the nature of the disease.

Dr. Tillman, Vere, states that it was 'almost entirely a question of food and clothing, as out of over nine hundred cases treated, not one case occurred among the coolie-children of the district.'

Tillman diagnosed the first case which he observed as one of meningitis, but later on he arrived at the conclusion that the meningeal hyperaemia was a secondary phenomenon, due to lung stasis and stasis in the venous system; the gastro-intestinal hyperaemia and irritation are considered phenomena of primary importance. The cause of the whole disorder was thought to be atmospheric. He reports on 144 cases observed, with only two deaths. He describes general malaise and sudden severe pain in epigastrium with violent vomiting, anxious expression, weak, rapid pulse, and slow and shallow respiration. The temperature is said to be often normal, but in most cases rising to 102 or 103, generally preceded by a rigor. Convulsions and death follow, unless treatment is resorted to, in which case the convulsions may be represented only by contractions of the feet, and develop no further.

The anatomical lesions found by Tillman were 'erythematous appearance' of the gastric mucosa, hyperaemia of liver, spleen, kidneys, and meninges, and hyperaemia and oedema of lungs.

The paper by Dr. Turton, Stony Hill, which is mentioned above, is also included in this report.

Dr. Calder, Santa Cruz, states that he has seen no cases exactly corresponding to the type of 'vomiting sickness' as described by his colleagues, but reports two cases in which vomiting was a salient feature, and in which he has performed post-mortem examinations. One was a child, the other an adult; pancreatitis, jejunal hyperaemia and haemorrhage, and petechiae in the stomach were observed in both cases.

It is obvious that various diseases may have been referred to in these reports. One difference is particularly striking between the views expressed in the reports of Bell, Tillman, and Turton, and those held by the other District Medical Officers. According to the latter, 'vomiting sickness' is an extremely fatal disease, whilst the type described by the latter is fairly benign, the mortality being as low as 2 per cent., or even less. It might be supposed that only

the latter had had the opportunity of seeing cases in the early stages, whilst the Medical Officers of the former group had always been too late, because of longer distances or for other reasons. As no specific treatment is indicated, it appears, however, entirely unintelligible that the character of the disease should have been so completely transformed by the measures described. Two possibilities remain, either that some observers have overlooked mild forms of the disease, or that others have included cases of a different nature. Neither possibility can be dismissed, but the latter seems perhaps the more probable. Many of the recovering cases would evidently, in the usual course of events, have been diagnosed as acute gastritis or gastro-enteritis, especially those with febrile temperatures and without collapse.

Dr. Ker concludes his report with the recommendation that special and systematic investigation into the question of 'vomiting sickness' should be made at an early date.

So far, in 1905, the entity of 'vomiting sickness' has not become established, a fact which must be borne in mind when discussing the following reports.

The Annual Report (1906) contains brief notes about 'vomiting sickness,' from various districts; it appears that the disease had been less prevalent and perhaps less malignant than in previous years. Two post-mortem reports, by Dr. Earle, are published; in both cases there was hyperaemia of meninges, lungs, liver, spleen, and kidneys. Petechial spots were observed in the brain substance, and subepithelial ecchymoses in the stomach. The descriptions are almost identical in the two cases, which are considered typical examples of the so-called 'vomiting sickness.'

In the 1907 Report it is stated that 'vomiting sickness' has been more prevalent and more severe than usual. In Clarendon some 80 or 90 deaths were actually reported, but many more are believed to have occurred. In Trelawny both adults and children were attacked, with fatal results. The need of a thorough investigation is again pointed out.

In 1908 'vomiting sickness' is said to have been little prevalent.

In 1909 the disease is reported present in Falmouth and Chapelton, but has evidently not been very prevalent.

In the 1910 Report exceptionally severe outbreaks are reported, and the following interesting table is given:—

'Number of deaths recorded as having been caused by Vomiting, Vomiting and Fever, Vomiting and Convulsions, etc.

Parish	Month							Total
	Dec., 1908	Jan., 1909	Feb., 1909	Mar., 1909	Dec., 1909	Jan., 1910	Feb., 1910	
St. Andrew	6	1	1	—	3	8	16	35
St. Catherine ...	9	4	3	2	22	10	1	51
Trelawny	15	38	13	7	7	12	9	101
Westmoreland ...	2	—	1	3	3	5	—	14
Clarendon	42	14	14	7	91	43	19	230
St. Thomas	—	1	—	—	4	8	1	14
Hanover	3	7	8	1	2	1	3	25
St. James	4	20	15	1	10	3	4	57
Manchester	7	13	3	3	47	19	1	93
								620

In the same Report the following statement is made by Dr. Ker: 'That many of these cases may be, and undoubtedly are, due to ackee poisoning, possibly cassava poisoning, worms, Meningitis, Bronchitis, Gastritis, and other diseases, there is little doubt, but beyond these there remains an uenxplained residue which needs explanation.'

In this connection the frequency of helminthiasis in children is mentioned; one District Medical Officer found, on administration of vermifuges, that out of 72 apparently healthy children only two expulsed no worms, whilst two passed more than a hundred ascarides each.

Cases and deaths are reported, in 1910, from many districts—it is evident that more attention is now being paid to the disease. Dr. Tillman reports 1,200 cases, the mortality not being stated.

Tillman modifies his earlier statement about coolie children: they do not escape altogether, but practically so; the different susceptibility is explained by the coolie children being better cared for than the children of the natives.

In the 1911 Report Dr. Ker abstains from discussion of the subject, awaiting the result of Captain Potter's investigations carried out during the corresponding financial year. Outbreaks of 'vomiting sickness' are reported from Stony Hill, Claremont, (St. Ann), St. Ann's Bay, Duncans (severe), Falmouth, Montego Bay, Newport, Crofts Hill, and Chapelton; in the report from Ulster Spring it is stated to have been 'practically absent.'

In 1912 no special reference to 'vomiting sickness' is made in the Annual Report, but two cases are stated to have died in Public Hospitals, the locality not being given.

II. RECENT OBSERVATIONS

It is obvious, from the data given in Section I, that a great many factors have been submitted for discussion as probable causes of 'vomiting sickness.' The existence of a separate morbid entity has been denied by several observers, but as a rule each observer has given his own view with regard to its nature, and these views have differed considerably—from helminthiasis to bronchitis, and from ackee poisoning to meningitis. The prevalent idea, however, has probably been that which was pronounced by Ker, that a group of unexplained cases remained when those were separated out which could be classified as well-known diseases. During the last year or so the discussion has taken on a somewhat more definite character.

Potter (1912), in a Colonial Office Report, published the first systematic investigation into the nature of 'vomiting sickness,' making the startling announcement that the majority of the fatal cases were yellow fever. This had probably been suspected by many, outside Jamaica, considering that the name of black vomit is given as a synonym of 'vomiting sickness' in Ker's summary, and remembering the existence in other West Indian Islands of the so-called 'fièvre à vomissements noirs des enfants,' by several authorities regarded as a form of yellow fever.

Potter does not declare that 'vomiting sickness' is identical with yellow fever; he agrees with other observers that the former name has been applied to a great many different diseases, and his report contains abundant evidence that this is the case. A large number of cases are included which have evidently nothing whatever to do with the type under discussion. Numerous cases are malarial, and others are otherwise definitely diagnosed. One group of seven cases, described in Appendix D to Potter's Report, shows very clearly the abuse, even of a most dangerous nature, to which an ill-defined name may lend itself. Five fatal and two non-fatal cases occurred in one hut, the District Medical Officer at first diagnosing 'vomiting sickness,' but afterwards suspicion of poisoning arose. One of the bodies was exhumed and arsenic was found in the organs. In spite of this evidence the investigation was not pursued, and the jury found that the cause of death was 'vomiting sickness.'

Eliminating from the discussion all these cases in which some other definite diagnosis can be made, Potter is of opinion that the remaining cases show the clinical characters and post-mortem appearances of yellow fever.

Before considering the question of yellow fever, Potter discusses the explanations previously advanced, and arrives at the conclusion that they are all unsatisfactory. With regard to cerebro-spinal meningitis he regards it as impossible that a large number of fulminant cases should develop without the simultaneous occurrence of other cases running a more prolonged course; besides, he says, in cases which had recovered, 'sequelae, such as paralysis, blindness and deafness should be expected,' and in fatal cases 'the post-mortem examinations would have shown definite inflammatory changes in the meninges.' The hypothesis of ptomaine poisoning has not been supported by symptomatological, nor by circumstantial evidence. The question of ackee poisoning as a cause of death—in some cases—is left open. Cassava poisoning is considered out of the question, as in that case the presence of prussic acid, the poisonous agent, would probably have been noticed.

Potter observed personally 73 cases, clinically or post mortem (the numbers run only to 70, but two different cases are both numbered 43, and the two cases in the additional section of

Appendix B were also seen by Potter); in addition he has collected a considerable number of observations from official reports. In many of the cases, both personal and from other sources, a definite diagnosis of malaria or some other affection was made, but a considerable number remains in which Potter suspects yellow fever, and in seventeen cases he feels certain of this diagnosis. In these seventeen cases the character of the stomach contents and the lesions of the gastric mucosa are certainly often suggestive of yellow fever, but the changes observed in other organs are usually so incompletely observed that the reader cannot possibly appreciate their nature and significance. It is very remarkable, and by no means in accordance with common experience in yellow fever, that the kidneys are often stated to be normal, and the urine free from albumin. The same objection which Potter makes against the assumption of the cases being cerebro-spinal meningitis can, with equal right, be made against a diagnosis of yellow fever: one would certainly expect that other cases occurred at the same time with more typical symptoms and a more prolonged course. To the reader of the report it is a great inconvenience that detailed descriptions are very seldom given, and that the findings recorded are not fully considered in the light of yellow fever pathology and epidemiology.

Potter's conclusions were at once contradicted by the Governor of Jamaica, in a letter to the Colonial Office. In support of his letter the Governor publishes a report by Scott (1912, 1), containing observations on cases of 'vomiting sickness,' with demonstration of meningococci in the spinal fluid. Later on, Scott recorded further observations in two Official Reports (1912, 2 and 1913, 1), and soon afterwards published a more detailed paper on the same subject (1913, 2).

In this last paper Scott summarizes his results. His first observations are on cases of meningitis of an ordinary type in which he found meningococci. Subsequently, he observed some cases of a fulminant type, showing the characters of 'vomiting sickness,' but occurring at a time of the year when this disease was usually not prevalent. All cases—five—occurred in one family. Investigation for meningococci was made in four out of the five cases, and the organism was found each time. In addition, meningococci

were found in the rhino-pharynx of a boy in the same family where the cases had occurred. Scott now followed up this line of investigation, and soon succeeded in finding the same organism in a considerable number of cases. These were all rapidly developing, but not all fatal; a considerable number belonged, undoubtedly, to the type of 'vomiting sickness.' Scott's paper deals with 52 cases, of which only a comparatively small number are personal observations, except for the examination of the spinal fluid, which he performed in 33 cases, the fluid either being obtained by himself or sent in by the Medical Officer in charge of the case. Meningococci were found in 23 cases, and similar cocci which could not be satisfactorily identified, in a few instances. In two cases spirochaete-like bodies were observed in the spinal fluid; both cases were of a rapidly developing type, and in one, at least, were meningococci also found. The spirochaete-like bodies were only seen on direct examination, but did not develop in cultures. The author leaves the question of their nature and importance open.

No anatomical lesions of any importance are mentioned in Scott's paper, except in the case of the meninges, and even in this case very few details are given. Little attention has evidently been paid to the pathology of the disease, which may, to a large extent, be accounted for by the fact that so few cases are personal observations.

Scott prepared vaccines from some of his cultures from the earlier cases, and used them later on in several mild cases and in a number of contacts—apparently with success. He fully realizes that vaccine treatment can have but little chance of success in the hyperacute cases, and, whilst recognizing the possible value of serum treatment, at the same time points out that its application meets with so many practical difficulties that it is impracticable in the majority of cases.

Scott's work furnishes a very definite basis for discussion. Since the appearance of his paper no further investigations have been published on the subject, but MacDonald (1913) has discussed and criticized Potter's paper, pointing out that he had failed to take up several important lines of investigation, and that no conclusive evidence had been brought forward in support of the diagnoses of yellow fever.

III. PERSONAL OBSERVATIONS

During my stay in Jamaica I observed 62 cases which were presented to me as cases of 'vomiting sickness,' and in which no other definite diagnosis could be made at once. A closer examination, however, revealed considerable differences, which make it necessary to divide the total number of cases into several groups for separate discussion. A brief summary of all the cases is given in the accompanying Table I; it has not been possible to include all symptoms and anatomical lesions, but, as it stands, I believe it conveys a fairly correct, general impression of the character of disease with which we have to deal in 'vomiting sickness.' Detailed histories of some of the cases—as far as they were obtainable—will be given further below when the various groups are discussed.

In addition to these personally observed and 'suspicious-looking' cases, I received from various colleagues observations which have not been included in the table, but some of them will be referred to later on. Nor have I included personal observations in which the symptoms at once suggested some other diagnosis than that of 'vomiting sickness,' unless the further course of events again appeared to bring this diagnosis in the foreground.

A few explanatory remarks are necessary in order to define more clearly the too brief headings of some of the columns in the table, and some indication of the pathological and bacteriological technique employed is also due.

First a general remark: the sign + indicates that the corresponding symptom was present, the sign −, that it is stated to have been absent. No sign means that no definite statement has been made in my notes; in this case, however, the symptom has usually been absent.

With regard to the clinical symptoms, it must be remembered that the majority of the patients did not come under medical observation during life. We therefore had to rely in these cases on layman's evidence, from which it, as a rule, was possible to elicit the presence or absence of symptoms as vomiting, convulsions, and coma. We found also that the statements with regard to the characters of the vomits usually appeared reliable. The vomited

Note.—Table I giving a synopsis of the cases will be found opposite p. 468.

matter was described, in the vast majority of cases, as watery or mucous, and sometimes it was said to have been greenish. With regard to black vomit, it was only described twice, and in two instances has its presence been queried. In one of the latter cases it was suspected because black stomach contents were found at the post-mortem examination, but nothing definite could be ascertained with regard to the appearance of the vomited matter. In the other case the grandmother of the patient, when telling the story of the child's illness, which had upset her considerably, to begin with mentioned black vomit, but afterwards denied emphatically to have said so, stating that the vomits had been absolutely colourless.

The column 'emaciation' has been inserted on account of the often-expressed opinion that 'vomiting sickness' is a disease chiefly or exclusively of emaciated or poorly nourished children. This opinion is emphatically contradicted by my experience, as the answers show. The sign + in this column does not exactly mean extreme emaciation, but simply a poorly nourished condition.

The column 'clinical character' gives the provisional diagnosis arrived at by the physician in charge and by myself, either from personal observation or from the information obtained from the parents or friends of the patient. The data given in the preceding columns are not always sufficient to bear out the diagnosis, which fact is explained by the peculiar circumstances under which we worked, especially that information often had to be obtained indirectly and from a class of people who were in many cases unable to give a definite answer to a concrete question, though a general impression was conveyed by their statements. In view of the vagueness of our opinion thus formed, the term diagnosis is avoided in the heading of this column.

In the column 'race,' bl. means black, and col. coloured; no cases were seen in whites, but the dictum that 'vomiting sickness' only occurs in the negro population was not confirmed, since two cases were observed in coolie children; the one case in a chinaman was of a different nature.

M. in the columns 18 and 19 means malarial parasites; P. means *Paraplasma flavigenum*.

The sign + in columns 19 and 20 means *Diplococcus meningitidis;* * indicates a growth of diplococci which differed from the

type of meningococci. The occurrence of other organisms, as staphylococci and B. coli, in a few cases, was regarded as accidental, and has not been tabulated.

In column 21, + indicates that albumin was found in the urine; in nearly all these cases hyaline or granular casts were also observed.

Most anatomical lesions are tabulated in accordance with the observations made at the post-mortem examination, but have, as a rule, been checked by histological observation. The statements with regard to 'inflammation' in liver, pancreas, and kidney refer exclusively to micro-cellular infiltration as observed by histological observation, and, consequently, do not not always correspond to the microscopical diagnosis of nephritis, etc., in the post-mortem reports; it is well known that the term 'acute parenchymatous nephritis' does not necessarily imply that cellular infiltration has already taken place.

Several organs, as thymus, thyroid body, pituitary body, ovaries, and others, have been entirely omitted from the table, as they never presented any lesions which appeared to have any essential bearing upon the question of 'vomiting sickness'; they were, however, automatically examined at the autopsies.

The post-mortem notes were dictated by me during the examination and written down at once by the Medical Officer or by an assistant.

The routine technique for histological examination was the following: Small fragments of the organs were fixed, at the autopsy, in sublimate alcohol (2 parts of saturated watery mercury bichloride solution, and 1 part of absolute alcohol), and in Flemming's strong osmic acid solution. Afterwards they were embedded in paraffin. Sections of sublimate-alcohol specimens were stained with iron-haematein, or with Friedlaender's or Delafield's haematoxylin, and counterstained with Hansen's aceto-picro-fuchsin solution, or with eosin. Other sections from the sublimate-fixed fragments were stained with Giemsa's solution or with Pappenheim's 'Panchrom,' according to the technique previously recommended (Seidelin, 1911, 1 and 2). In the case of the Flemming technique sections were stained with safranin.

The organs usually preserved for histological observation were

liver, pancreas, kidney, spleen, lymph nodules, and myocardium. Stomach, duodenum, adrenals, brain, and spinal cord were frequently examined, and other organs occasionally, according to special indications.

Examination for blood parasites was made in films of peripheral blood and heart blood, and from organs as spleen, liver, kidneys, and surface of brain. Microscopical examination was also made of the centrifugalization-deposit of the spinal fluid.

Cultures were made from the fluid obtained by lumbar puncture, in vivo or post mortem, or from the lateral ventricles of the brain, at the autopsy, and in many cases from the heart blood. Ascites-agar (nasgar) was the medium employed, and in addition to this, in some cases haemoglobin-agar or nutrient agar. Cultures were considered negative if no colonies developed within a week.

A positive diagnosis of *Diplococcus meningitidis* was made, if small, pale, greyish, semi-transparent colonies developed, consisting of Gram-negative diplococci, which produced acid in glucose, maltose, and galactose media, or changed at least one of these, but produced no change in other sugars. Similar organisms, which differed from the typical meningococcus in one or several particulars, were frequently met with, and will be the object of special discussion.

IV. DISCUSSION OF PERSONAL OBSERVATIONS

In order to discuss the cases tabulated in the preceding section, it will be convenient to state the characteristics which would appear to justify a diagnosis of 'vomiting sickness,' with the exclusion of other diseases. This means the provisional recognition of 'vomiting sickness' as a morbid entity, but the possibility must be left open that this entity may fall to pieces afterwards if it is found on further investigation that the cases may be classified under other headings. As characteristic symptoms I would regard: sudden onset; rapid development, the disease running its course to recovery or death in less than twenty-four hours, as a rule, in children, and in a few days in adults; vomiting; convulsions; coma; absence of fever, or only low fever; collapse; in fatal cases, anatomical lesions pointing to an acute intoxication, or hyperacute generalized

infection; negative result of the investigation for well-known pathogenic organisms.

This provisional definition having been laid down, the cases fall naturally into various groups. These groups will first be briefly discussed, and particulars of typical cases afterwards be given as illustrations of each group.

In the first group, A, all cases are included which correspond more or less closely to the above definition. The cerebro-spinal fluid has been examined in all cases, microscopically and by cultural methods; the result was always negative, as far as the *Diplococcus meningitidis* was concerned, but in some cases other microbes were found, especially *Diplococcus crassus* and other meningococcus-resembling diplococci. The blood was examined as far as possible, and in the cases where such examination was made the result was negative with regard to both malarial parasites and *Paraplasma flavigenum;* in all other cases the diagnoses of malaria and yellow fever were considered out of question, being unsupported or not sufficiently supported by the clinical and (or) pathological evidence. This group will, of course, form the principal object for the following general discussion; in this place it shall only be pointed out that the most constant and most remarkable pathological changes were: fatty metamorphosis of liver, kidneys, and other organs; necrobiotic changes of epithelia in pancreas, kidneys, and liver; swelling and hyperaemia of lymph nodules; hyperaemia of many organs, including the meninges, and a tendency to haemorrhages; widespread oedema of the connective tissues. The following cases belong to this group: Nos. 9, 14, 15, 16, 34, 37, 40, 41, 42, 46, 49, 52, 56, 59, 60, and 61; altogether 16 cases. Several cases, provisionally classified under other headings, may also belong to this group; case 58, for instance, closely resembles the cases here included, and would have been classified accordingly but for the reason that a few malarial parasites were found in the heart blood, an occurrence which is very likely to be quite casual.

Notes from the cases in group A are given below, some in full as types, others in abstract.

In the first case recovery took place; this case is, I believe, typical of the mild form of 'vomiting sickness.'

Case 9. A.S., 3 years, brown, 4, Chancery Lane, seen with Dr. Dryden in the Public Hospital, Kingston. January 21st, 1913.

Admitted in a weak condition; started vomiting at about 8 a.m., two hours before admission. Vomited several times in hospital; the vomiting consisted, as before, of abundant quantities of watery, frothy material, and took place without any effort. The impression was most nearly that of an acute obstruction of the pylorus. The child was stimulated, and reacted slowly in the course of the day, without any specific treatment. He appeared well the following day, and was discharged after two days, still somewhat weak and pale, but this seemed habitual.

Cerebro-spinal fluid was taken on the day of admission, at 2 p.m. Result: Temperature normal. Pulse frequent, slightly irregular, soft. No abdominal tenderness; no diarrhoea. No meningeal symptoms.

There is a suspicion that the child had eaten 'physic nut' before taken ill, but nothing definite is known, and the clinical picture did not correspond to that described in cases of poisoning with *Jatropha curcas*.

The following three cases, in three brothers, were seen by Dr. Joslen, of Annotto Bay. The first patient was also examined by me, and in the two other cases I performed the post-mortem examinations with Dr. Joslen.

Case 14. A.N., 12 years, brown, born in Jamaica and has always lived there. He was seen by Dr. Joslen at Evandale, St. Mary, where he lived in a poor hut, with a leaking roof, in the bush, together with his father and two brothers. The brothers were taken ill at the same time as A.N. (cases 15 and 16); the father was unwell for about a day, complaining of fullness in the chest.

A.N. was taken ill, suddenly, on January 26th, 1913, at 10 p.m., with vomiting, accompanied by considerable retching, slight epigastric pain, and slight headache. He soon became weak and drowsy. Thus he was found on January 27th, at about 5 p.m., and next day he was taken to the Hospital in Annotto Bay, where he arrived at 4.20 p.m. There I saw him the same evening, with Dr. Joslen.

January 27. Temperature 98·4° F. Pulse 120, regular, weak. Respiration 16, regular.

January 28, afternoon: temperature 98·4° F.; pulse 120. Evening: temperature 99° F.; pulse 88, regular, soft. No muscular rigidity; Kernig's symptom absent. Slight abdominal pain, corresponding to descending colon, but no tenderness. No enlargement of liver or spleen. Heart dullness and heart sounds normal. No sign of lung affection. The patient has no lice and no ticks.

January 29. Temperature normal; pulse 84, slightly irregular. The patient has slept well, feels well, and is hungry.

The patient recovered.

Lumbar puncture was performed in the evening of January 28; about thirty small drops of clear fluid were taken into two nasgar tubes, which remained sterile.

Blood smears were taken at the same time: they contained no parasites.

Case 15. C.N., 9 years, brown, born in Jamaica, and has lived there always. Evandale, St. Mary. Brother to Cases 14 and 16. Good health until about 10 p.m., January 26th, 1913, when he suddenly started vomiting. He became weak and drowsy, and died at about 5—6 p.m., January 27th.

Symptoms as in Case 16.

Post-mortem examination at 8 p.m., January 28th, 1913. Body of poorly nourished child. No jaundice. No rigor.

Lumbar puncture gives about thirty drops of clear fluid. *Spinal membranes*, both dura and pia, hyperaemic. Spinal cord shows no microscopical lesions.

Brain preserved unopened; the external aspect is normal. Dura mater cerebralis normal, pia considerably hyperaemic.

Hypophysis cerebri of normal size and aspect.

Abdominal cavity contains no fluid. Peritoneum of normal aspect. Abdominal organs normally situated.

Spleen: 18 × 11 × 3 cms. Capsule smooth, transparent. Colour reddish grey. Consistence diminished, slightly friable, not soft.

Right *kidney:* 9 × 4½ × 2 cms. Capsule easily detached. Surface smooth, without hyperaemia and haemorrhages. Consistence normal. Cortical substance pale. Slight hyperaemia of pyramidal bases.

Left kidney as right.

Mucosa of renal pelves, ureters, and bladder normal.

Genital organs normal.

Liver: 30 × 14 × 7 cms. Left lobe very long, which accounts for the excessive length of the organ, which is not essentially enlarged. Capsule smooth, transparent. Colour greyish red, with some paler, but not distinctly yellowish patches. The tissue is more uniformly grey. Consistence normal.

Pancreas: 12 cms. long, slightly hyperaemic; consistence soft.

Suprarenal capsules of normal aspect.

Pleural cavities contain no fluid. There are extensive, but delicate, adhesions of right pleura and similar, but less developed, of the left. *Lungs* hyperaemic in postero-inferior portions, contain air throughout. No infiltrations, no oedema, no infarcts. Slight anthracosis.

Bronchial lymph nodules slightly enlarged, anthracotic, and some of them fibrous, but no definite tuberculous phenomena are met with in lymph nodules or in lungs.

Larynx, *trachea*, and *bronchi* normal, except that frothy fluid escapes from the bronchi.

Tongue slightly coated.

Tonsils enlarged and the right considerably hyperaemic.

Oesophagus normal.

Thyroid body normal.

Pericardium contains no fluid. No pericardial, nor endocardial haemorrhages. Heart-conus: 6 × 8 × 2½ cms. Left ventricle fairly well contracted. Endocard and all valves normal. Myocard pale, of slightly diminishing consistence, shows fine yellowish stripes.

The *stomach* contains a considerable amount of brownish fluid, apparently not haemorrhagic, but corresponding to intestinal contents. The mucosa shows considerable hyperaemia, and on the anterior surface, near to the lesser curvature, are observed several petechiae.

The *duodenum* shows hyperaemia, but no haemorrhages. At a distance of about 2 cms. below the pylorus an ulcer is observed, which is of oval shape, 8 mms. long and 6 mms. wide, and is situated on the posterior wall. The ulceration has penetrated the mucous and muscular coats, and the bottom is formed only by the retro-duodenal tissues. The borders of the ulcer are slightly infiltrated, but there is no overlapping of the edges. The *jejunum*, *ileum*, *colon* and *appendix* without pathological phenomena.

Bile ducts and *gall-bladder* free from stones and obstructions; the gall-bladder contains greenish bile.

Numerous ascarides are present, about 35 in the small intestine, and one in the stomach. In the coecum a few *Tricocephali* are present.

Anatomical diagnoses: Hyperaemia meningum. Hyperplasia lienis. Hypostasis pulmonum. Metamorphosis adiposa hepatis. Pancreatitis parenchymatosa acuta. Sequelae pleuritidis duplicis. Gastritis acuta cum petechiis. Ulcus duodeni. Helminthiasis intestinalis.

The histological examination of the organs was of no particular interest, as post-mortem changes were marked. It may be mentioned that sections of the *liver* showed intense fatty change, which was irregularly distributed throughout the lobules; the tissues were anaemic, where fatty change was most marked, in other places capillary hyperaemia was observed. The *pancreas* showed marked oedema of the stroma and partial necrobiosis of epithelia. The *myocard* showed fragmentation and oedema of stroma.

A short, Gram-negative bacillus developed in the nasgar-tubes, inoculated with spinal fluid; no cocci.

Case 16. C.N., 5½ years, brown, born and lived in Jamaica, Evandale, St. Mary. Brother to A.N. and C.N., Cases 14 and 15.

The patient was seen by Dr. Joslen, Annotto Bay, January 27th, 1913, and the post-mortem examination performed by me on January 28th.

Apparently good health until January 26th, 10 p.m., when he suddenly started vomiting, and went on vomiting until death. Drowsy, listless and weak. Died at 5—6 p.m., January 27th.

No desire for food, but thirst. No chilliness, rigor, convulsions. No eye symptoms. Skin natural, no eruption, no jaundice. No fever. No lung symptoms. No headache. Perhaps slight cardialgia; considerable retching. No delirium, irritability, paralysis, rigidity, nor retraction of neck.

Post-mortem examination at 8 p.m., January 28th, 1913.

Body poorly nourished. Rigor mortis is present. No lice are observed. There is no jaundice. Superficial ulcerations are observed on both lower extremities.

Lumbar puncture gives 15 drops of blood-stained fluid.

The spinal *meninges*, both pia and dura, are hyperaemic, and the spinal cord is also somewhat hyperaemic.

The cerebral dura is normal, but the pia is considerably hyperaemic, especially on the convex surface of the brain. There is no fibrinous exudation. The lateral ventricles are not distended. There is no hyperaemia, and no other pathological lesions of the brain substance.

The *hypophysis cerebri* and the *thyroid body* are normal.

The *larynx*, *trachea*, and *bronchi* are normal. No pleural fluid, nor adhesions.

The *lungs* show hyperaemia in their postero-inferior portions, but neither oedema, nor infiltrations, nor infarcts; there is slight anthracosis. The bronchial lymph nodules are slightly enlarged and anthracotic.

The *heart* conus is 5 × 6 × 3 cms. The left ventricle is well contracted. The pulmonary artery contains no thrombi. All the valves are normal, as is the whole of the endocardium, and the pericardium. The myocardium is pale, otherwise normal. The pericardium contains a few cubic centimetres of clear fluid.

The *inguino-crural lymph nodules* are slightly enlarged, the axillary not enlarged.

The abdominal cavity contains no fluid; the peritoneum is of normal aspect. The abdominal organs are normally situated, with the exception of the kidneys. The sigmoid flexure is considerably distended with faeces.

The *mesenteric lymph nodules* are slightly enlarged ; they show no haemorrhages, and no signs of tuberculosis.

The diaphragm reaches on the right side the 5th, on the left side the 4th rib.

The *spleen* is 11 × 7 × 2 cms. Capsule smooth, transparent ; colour dark greyish red ; no haemorrhages ; consistence soft ; follicles grey, somewhat prominent. A supernumerary spleen is found in the gastro-splenic ligament.

The *suprarenal capsules* are normal.

The *kidneys* are united at their inferior extremities in the shape of a horse-shoe, with two separate pelves on the anterior surface. The capsule is easily detached. The surface is smooth, without any vascular distension. The renal tissue is normal ; there is slight hyperaemia of pyramidal bases. The mucosae of the renal pelves, ureters and bladder are normal. The bladder contains a considerable quantity of pale urine. The genital organs are normal.

The *liver* is 18 × 12 × 5 cms. The capsule is smooth and transparent ; colour uniformly red ; substance reddish grey without haemorrhages, and with no yellow patches. Consistence normal.

The *pancreas* is 10 cms. long, hyperaemic, and of diminished consistence.

The *stomach* contains alimentary substance and some mucus, but no blood. There are no haemorrhages in the mucosa.

Oesophagus, normal. *Tonsils* without signs of acute inflammation. *Tongue* slightly coated.

The intestines present no pathological changes. A large number of ascarides are present, but no other parasites.

The *bile ducts* are patent ; the gall bladder contains a considerable amount of greenish bile ; mucosa normal.

Anatomical diagnoses : Hyperaemia meningum. Hypostasis pulmonum. Lymphadenitis bronchialis, mesenterialis et inguinalis. Splenitis acuta. Pancreatitis parenchymatosa acuta. Lien accessorius. Symphysis renum. Helminthiasis intestinalis.

As in the foregoing case, post-mortem changes interfered with the histological examination. Sections of an inguinal *lymph nodule* showed marked hyperaemia and some lymphocyte-infiltration of the capsule. Sections of the *spinal cord* showed hyperaemia, but no cellular infiltration of the pia, and, apparently, oedema of pia and cord.

Short, Gram-negative bacilli develop in the nasgar tubes, inoculated with the spinal fluid ; no cocci.

The following is an example of vomiting sickness in a child of East Indian race, though, I believe, born in Jamaica. In this case, the presence of jaundice aroused suspicion of yellow fever, but after examination of stomach and liver this diagnosis was entirely dismissed.

Case 34. R.S., 4 years, coolie child, Windsor Park, St. Catherine, seen, after death, with Dr. A. G. Curphey, at the Hospital, Spanish Town, on February 6, 1913.

The child was admitted this morning, at 10 a.m., in a dying condition. He was said to have been taken ill suddenly the previous day, February 5, at 3 p.m., with vomiting. The vomits consisted of frothy, watery fluid, and were repeated the following morning. The child was crying and restless, but no shrieks were noticed. He had been healthy until this illness. In the hospital clonic convulsions were observed with intervals of about five minutes. There was no strabismus ;

no rigidity of neck ; no Kernig's symptom. Temperature 101·4°. The pulse was rapid, about 140, weak, regular. Frothy fluid escaped from the mouth, no vomiting took place in the hospital. A yellow stool was obtained after enema. The child died at 11.15 a.m. (Notes by Dr. Curphey, as I arrived not until about 2.30 p.m.).

The mother reports that *another child* of hers died suddenly on February 1, after having vomited watery, frothy fluid.

One child alive and in good health.

Post-mortem examination, at 3 p.m., on February 6, about four hours after death. Well nourished body. Rigor present. Slight jaundice. Slight enlargement of lymphatic nodules in neck, axillae, and inguina.

Lumbar puncture gives clear fluid, of which eight drops are taken in nasgar tube, and two smears are made. Spinal cord not examined. *Dura mater cerebralis* normal, *pia* slightly hyperaemic in posterior part of convex surface. No distension of lateral ventricles. *Brain* substance macroscopically normal throughout.

Hypophysis cerebri and *thyroid body* of normal size and aspect.

The *pleural cavities* contain no fluid, and there are no pleural adhesions. The *lungs* show slight hyperaemia of postero-inferior portions, but are otherwise normal. The bronchial lymph nodules are slightly enlarged and anthracotic. The bronchi, trachea, and larynx are normal.

The *pericardium* contains no fluid, and shows no petechiae. The heart is of normal size. The pulmonary artery is free. The endocard and all the valves are normal, the myocard is pale, greyish, of normal consistence.

The *peritoneum* is of normal aspect, and contains no fluid. Abdominal organs normally situated.

The *tongue* is slightly whitish coated. The tonsils are not enlarged. The oesophagus is normal. The *stomach* shows normal conditions ; there is no hyperaemia of the mucosa, and no haemorrhages. The *duodenum* shows considerable, and the *jejunum* slight swelling of lymphatic follicles ; no other abnormal conditions of intestinal tract. The bile ducts are patent ; the gall bladder contains a small quantity of greenish bile.

Liver : 20 × 11 × 5 cms. Capsule smooth, transparent ; surface reddish, with grey and yellowish patches ; the consistence is normal in the red portions, but diminished in the yellowish ones. The structure of the organ is homogeneous, without distension of vessels.

The *pancreas* shows slight hyperaemia, but is of normal size and consistence.

The *spleen* measures 6 × 4 × 1½ cms. Its capsule is smooth and transparent, its colour dark greyish red, and its consistence normal. The follicles are grey and prominent.

There is a slight hyperaemia of the left *suprarenal capsule*, but not of the right one, and both organs are otherwise normal.

The left *kidney* is 7 × 4 × 2 cms. The capsule is easily detached, the surface is smooth, dark, and shows capillary, but not venous, hyperaemia. The renal substance is dark red, without haemorrhages, and without yellow patches. The right kidney shows conditions similar to those of the left, except that the colour is slightly more pale.

The mucosae of renal pelves, ureters, and bladder are normal. The bladder contains about 50 c.c. of clear, yellow urine.

The *genital organs* are of normal aspect.

The *urine* is pale, clear, acid, contains a considerable amount of albumen, and a few casts, besides epithelial cells ; it contains no sugar, no bile pigment, no erythrocytes, and no leucocytes.

Anatomical diagnoses: Hyperaemia piae matris cerebri l.g. Hypostasis pulmonum l.g. Duodenitis follicularis. Jejunitis follicularis l.g. Lymphoadenitis mesenterialis, bronchialis, cervicalis, axillaris et inguinalis simplex. Metamorphosis adiposa hepatis. Hyperaemia pancreatis, glandulae suprarenalis sinistrae, et renum. Icterus.

Histological examinations:—

Liver: Marked capillary hyperaemia, slight microcellular infiltration of periportal connective tissue. No necrobiosis, and only very slight vacuolization of cells.

Pancreas: Marked venous and capillary hyperaemia, slight oedema of stroma, small patches of necrobiotic cells.

Spleen: Diffuse hyperaemia.

Kidney: Hyperaemia, which is very irregularly distributed, and often very intense; in a few places some interstitial leucocyte-infiltration; slight vacuolization of basal portions of cells in some of the convoluted tubules; in the same kind of tubules necrobiotic changes are common, with granular appearance of the protoplasm and more or less advanced karyolysis.

Duodenum: Enormous hyperplasia of Brunner's glands which not only increase the thickness of the submucosa, but also occupy large parts of the mucosa, even dislocating and compressing the Lieberkühn's crypts, and in some places reaching the free surface. There is also considerable hyperplasia of the lymphoid follicles and diffuse lymphoid infiltration of the mucosa.

Axillary lymph nodule: Diffuse hyperaemia and slight oedema of stroma.

Brain: Slight hyperaemia, no microcellular infiltration.

Spinal fluid: Very few cells, chiefly erythrocytes; some mononuclears, and a few polymorphonuclears.

Microbiological examination:—

Heart-blood: No growth.

Spinal fluid: Growth of a diplococcus, which forms large groups. It decolourizes with some difficulty by Gram's and Claudius's methods. It produces acid in glucose, maltose, galactose, lactose, and saccharose media.

Spleen: Growth of diplococcus identical with the one in the spinal fluid.

No parasites are found in smears from heart blood, liver, spleen, and spinal fluid.

In the following case there was no vomiting, but otherwise it appears to belong to the type of 'vomiting sickness.' There were no signs of meningitis or yellow fever, clinically or anatomically. No growth was obtained from the spinal fluid, and none in nasgar tubes from heart blood, but on haemoglobin-agar inoculated with heart blood there was a growth of diplococci, otherwise similar to meningococci, but showing the striking difference, that the colonies, both on haemoglobin-agar, and in subcultures or nasgar, were of a saturated whitish appearance.

Case 40. A.M.L.Y., 2½ years, black, Barbican, near Cherry Garden Gate, seen on February 12, 1913, with Dr. Croswell.

The child has enjoyed good health until this morning. She awoke at 6 a.m., apparently well, asking for water. Soon after, however, she complained of pain

in her stomach, and was seized by convulsions, apparently a kind of slow clonic contractions. She has suffered from no previous disease; in particular, she has had no cough, no fever, and no symptoms of worms. She was seen by Dr. Croswell at 8 a.m.; she was then comatose, and there was complete muscular relaxation. There was no abdominal retraction, no rigidity, and no Kernig's symptom. Temperature 97·8° F. No jaundice. The action of the heart was fairly strong. Injections of strychnin, digitalis, and nitroglycerin were given.

The child was in a dying condition, when I arrived at 10.45 a.m. The respiration was superficial, the pulse weak, but regular; it became immediately filiform, and could not be counted, but was not very frequent. There were no convulsions, and no rigidity, especially not of the neck, and no Kernig's symptom. The arms were faintly contracted in a semi-flexed position, but immediately afterwards became relaxed. The teeth were closed. There was grinding of the teeth. The child gave a faint shriek, and died at 10.50 a.m. At the moment of death, urine was passed in considerable quantity. The abdomen was considerably distended and this distension was said to have developed during the last ten minutes. There was no enlargement of liver or spleen.

About an hour after death spinal fluid and heart blood were taken in nasgar and haemoglobin-agar tubes, and smears were made.

Post-mortem examination (with Dr. Edwards, at 11 a.m., on February 13, about twenty-four hours after death). Well nourished, healthy looking body.

Dura mater cerebralis, and, particularly, spinalis considerably hyperaemic; pia mater slightly hyperaemic, mostly pronounced on convex surface of the brain and on the lumbar portion of the cord. No inflammatory exudation, and no free fluid in the lateral ventricles. The brain and spinal cord are of normal appearance.

The *inguinal*, *axillary*, and *cervical lymph nodules* are slightly enlarged, and intensely hyperaemic.

The abdominal cavity contains no fluid. The peritoneum is of normal aspect. The abdominal organs are normally situated. The intestines are considerably distended. The diaphragm reaches the fourth intercostal space on both sides.

The *spleen* measures 7 × 5 × 2 cms. The capsule is smooth and transparent, the colour dark red and grey. The follicles are grey and very prominent, the consistence normal.

Suprarenal capsules normal.

Right *kidney:* 6 × 4 × 1½ cms. Capsule easily detached; surface smooth, with venous and capillary hyperaemia; cortex hyperaemic, with yellowish grey patches, in its central portions; pyramids pale. Left kidney shows the same conditions as the right.

Renal pelves, *ureters*, and *bladder* of normal appearance. The bladder contains a small quantity of pale urine.

Genital organs normal.

Mesenteric lymph nodules considerably enlarged and slightly hyperaemic.

Liver: 23 × 13 × 4 cms. Capsule smooth, transparent; colour dark purple; substance uniformly reddish grey; consistence normal.

The *pancreas* is hyperaemic and of soft consistence.

The *stomach* contains a considerable quantity of bile-stained mucus. The mucosa is hyperaemic, but without haemorrhages.

The *duodenum* is hyperaemic. The jejunum shows a few hyperaemic patches and slight swelling of both solitary follicles and Peyer's patches. The swelling of the patches increases considerably in the ileum, especially in its terminal portion and is here accompanied by hyperaemia. The colon shows throughout its whole

length follicular swelling and on the top of each swollen follicle a black, minute spot. There are no parasites in the intestinal tract, except one small ascaris in the ileum ; in particular no parasites are seen corresponding to the swollen follicles in the colon. The bile ducts, gall bladder, and pancreatic ducts are of normal aspect. The gall bladder contains a small quantity of greenish bile.

The *oesophagus* is normal.

The *tonsils* are considerably swollen and hyperaemic, with haemorrhages in the tissue.

The *tongue* is covered, on the whole surface, with whitish fur.

The *larynx* is slightly hyperaemic, the trachea and the larger bronchi more pronouncedly so, and in the smaller bronchi, the mucous membrane is both swollen and hyperaemic, whilst there is an abundant mucous secretion. There is a pronounced hyperaemia of the postero-interior portions of both *lungs*, whilst the anterior portions are markedly pale.

The *bronchial lymph nodules* are slightly enlarged and hyperaemic, as well as the mediastinal. There are no signs of tuberculosis in either lungs or lymph nodules.

The *pleurae* are normal.

The *pericard*, *endocard*, and *valves* are normal. The *myocardium* is very pale.

The *hypophysis cerebri* and the thyroid body are normal.

The *thymus* measures $5 \times 4 \times \frac{1}{2}$ cm. ; it is of normal appearance and consistence.

Anatomical diagnoses : Lymphadenitis acuta. Tonsillitis haemorrhagica. Enteritis follicularis. Colitis follicularis et pigmentosa. Lymphadenitis mesenterialis m.g. Hyperaemia ventriculi, renum et meningum. Pancreatitis acuta parenchymatosa. Splenitis acuta follicularis. Hypostasis pulmonum. Bronchitis capillaris. Bronchitis, tracheitis et laryngitis incipiens. Anaemia myocardii. Helminthiasis intestinalis (*one* ascaris).

Urine : Contains small quantity of albumen and some hyaline casts.

Histological examination :—

Axillary and *cervical lymphatic nodules :* intense hyperaemia, venous and capillary. Moderate oedema of stroma. *Mesenteric lymphatic nodules :* oedema of stroma, but no hyperaemia.

Spleen : Intense diffuse hyperaemia.

Liver : Intense diffuse hyperaemia, moderate fatty changes equally distributed throughout the lobules, slight necrobiotic changes.

Microbiological examination :—

Nasgar tubes inoculated with *spinal fluid* and *heart blood* remain sterile, except one in which one colony of short Gram-negative bacilli develops.

Haemoglobin agar tubes inoculated with spinal fluid remain sterile, but not those inoculated with *heart blood*, in which saturated white colonies develop, consisting of Gram-negative cocci, mostly diplococci which grow abundantly, also in nasgar, and which ferment glucose, maltose, and galactose, but not lactose and saccharose.

The heart blood contains microscopically no organisms ; smears from liver, kidney, spleen and mesenteric lymph nodules contain various types of bacteria and those from liver and kidney contain also small diplococci. Smears from bone marrow contain no organisms.

Case 41. M.A.B., 3 years, black, Whitehall, with Dr. C. R. Edwards, Half-way Tree.

Seen alive by Dr. Edwards, not by me. Previous health good. Taken ill in the morning of February 13, 1913, with vomiting and convulsions; died at 11 a.m. on the same day.

Post-mortem examination, at 11 a.m., on February 14. Body fairly well nourished. No cutaneous affections, nor parasites. Rigor present. No jaundice. On the skin of the back a number of irregular, black patches, as from burns, and on the left hip a bulla, 5 × 2 cms.

Cervical, *axillary*, and *inguinal lymph-nodules* swollen and intensely hyperaemic.

Spinal fluid taken after death by Dr. Edwards, clear; only a small quantity was obtained. On opening the spinal canal the fluid was found somewhat thickened, slightly gelatinous. Cerebral dura shows slight, and spinal dura moderate hyperaemia. *Spinal* and *cerebral pia* shows considerable hyperaemia, mostly pronounced on the convex surface of the brain. No distension of the lateral ventricles. Cerebral and medullar substance normal.

Hypophysis cerebri normal.

Diaphragm on the right side at fourth, and on the left at fifth intercostal space.

Pleurae without fluid and adhesions. The *Lungs* are slightly hyperaemic, chiefly in postero-inferior portions. They contain several small, haemorrhagic infarcts, and numerous petechiae are seen on the pleura of the right inferior lobe. There is slight oedema of the inferior lobes of both lungs.

The *bronchial lymph nodules* are slightly enlarged and slightly hyperaemic.

The smaller bronchi show hyperaemia and swelling of their mucosa and considerable mucous secretion; similar conditions obtain in the larger bronchi, trachea, and larynx.

Thyroid body normal.

Pericardium, *pulmonary artery*, and *aorta* normal. Epicardium hyperaemic. Left ventricle well contracted, forms apex. Heart conus 5 × 6 × 2 cms. Endocard and valves normal. Myocard pale, of slightly diminished consistence.

Peritoneum normal, contains no fluid, abdominal organs normally situated. Appendix occupies a posterior, lateral, ascending position.

Mesenteric lymph nodules considerably enlarged and slightly hyperaemic.

Spleen: 7 × 5 × 2 cms. Capsule normal; colour dark purple, with grey spots; follicles slightly prominent, grey; consistence normal.

Suprarenal capsules normal.

Right *kidney:* 8 × 4 × 2 cms. Capsule easily deatched. Surface dark, with capillary hyperaemia; cortex hyperaemic, with yellowish grey patches in central portions. Pyramids pale. Left kidney shows the same characters.

Mucosa of renal pelves and ureters hyperaemic. The bladder contains a considerable quantity of pale yellow urine, of which some part is collected; the mucosa is normal.

The *oesophagus* is hyperaemic. The *stomach* contains a small quantity of a alimentary fluid; the mucosa shows slight hyperaemia. The *duodenum* shows considerable hyperaemia and some petechiae. The *jejunum* shows numerous hyperaemic patches and a few ecchymoses in its upper portions. *Ileum* pale, with considerable swelling, but no hyperaemia of Peyer's patches. *Appendix* 10 cms. long, normal. The *large bowel* shows a few hyperaemic patches, is otherwise normal. The jejunum, ileum, and colon contain an enormous number of guava seeds; also a few in appendix. One Tricocephalus in coecum. No other parasites.

Bile ducts and gall bladder are patent and of normal aspect.

The *liver* measures 22 × 11 × 4 cms. Capsule normal ; colour purple grey, with a few yellowish patches ; consistence and structure normal.

The *pancreas* is hyperaemic and of dimished consistence.

The *tongue* is covered on its anterior portion with a whitish fur, on the posterior part of its surface with a dark and slightly haemorrhagic coating.

The *tonsils* are enlarged, hyperaemic, and show in their tissue haemorrhages and purulent infiltration.

The *thymus* measures 5 × 3 × ½ cms., and shows normal aspect.

The *urine* is pale, clear, contains a small quantity of albumin and a few granular casts ; no sugar, no bile pigments.

Anatomical diagnoses : Poly-lymphadenitis acuta. Sequelae combustionis. Hyperaemia meningum. Hyperaemia et infarcti haemorrhagici pulmonum. Petechiae pleurae dextrae. Laryngitis, tracheitis, bronchitis et bronchitis capillaris catarrhalis. Nephritis parenchymatosa acuta. Hyperaemia ventriculi et intestinorum. Ecchymoses duodeni et jejuni.

Histological examination :—

Liver : Intense hyperaemia of a diffuse character ; marked fatty change ; slight necrobiosis and slight microcellular infiltration of stroma.

Pancreas : Almost complete necrobiosis of cells, the nuclei not staining with haematein, and the cell limits badly defined.

Kidney : Intense necrobiosis of epithelia of convoluted tubules, the protoplasm of which is granular and the nuclei poorly stained or entirely unstained. Glomeruli and straight tubules fairly well preserved.

Myocardium : No changes observed in sections.

Lymph nodule : Diffuse hyperaemia ; patches of necrobiosis.

Spinal cord : Pia mater shows oedema and patches of microcellular infiltration.

Tonsil : Localized necrosis and haemorrhages.

Microbiological examination :—

Large Gram-positive bacteria develop in cultures on nasgar from the spinal fluid ; similar bacteria are seen in smears from the spleen, and in sections from various organs. No diplococci, in smears, or cultures. Culture-tubes (nasgar and haemoglobin-agar) inoculated with heart blood remain sterile.

In the following case, the clinical symptoms suggested a diagnosis of meningitis ; this diagnosis, however, was not confirmed anatomically or microbiologically ; the case may provisionally be referred to as one of ' meningismus.'

Case 46. E.B., 2½ years, black, Rodney Street, Admiral Town (near Kingston), seen at Public Hospital, Kingston, with Dr. Thomson.

Sudden onset of convulsions at about 7 a.m., February 17, 1913. No vomiting. Admitted to hospital at 9.45 a.m. ; temperature 98° F. ; patient unconscious ; pupils dilated. Limbs rigid ; head retracted, marked opisthotonus. Kernig's sign present, most marked on the right side.

Lumbar puncture made (Dr. T.) : Water-clear fluid escapes under considerable pressure.

When examined later on, by Dr. T. and myself, at 12.30 p.m. the limbs are absolutely limp, and there is only a faint indication of Kernig's sign on the right side, whilst it is entirely absent on the left. Still later, it is absent on both sides. Pulse fairly strong, but very irregular, about 100.

Several convulsions occurred in the hospital, consisting chiefly in tonic spasms, and the child died at 4.30 p.m.

Post-mortem examination, at 11.30 a.m., on February 18, nineteen hours after death. Body well nourished. No skin affections, nor parasites. No rigor. No jaundice.

Cervical, axillary, and *inguinal lymph nodules* slightly enlarged and hyperaemic.

The *subdural space* contains a considerable amount of serous fluid at the basis of the cranium, and fluid is seen escaping from the spinal canal. Slight hyperaemia of cerebral dura ; spinal dura normal. Pia slightly hyperaemic, mostly so on the convex surface of the brain, and on the lumbar portion of the cord. No fibrinous exudation. The lateral ventricles are not dilated, and contain only a few drops of clear fluid. Brain and cord substance normal.

Hypophysis cerebri somewhat hyperaemic, not enlarged.

Pleurae normal. Both *lungs* contain a few small haemorrhagic infarcts, are otherwise perfectly normal, without hypostasis, and without anthracosis. Bronchi, small as well as larger, trachea, and larynx, normal. Bronchial lymph nodules moderately enlarged and hyperaemic, not anthracotic.

Thymus : 5 × 2 × 0·3 cms., of normal appearance.

Thyroid body normal.

Pulmonary artery and *aorta* normal. *Pericardium* contains a few c.c. of clear fluid. Left ventricle well contracted, forms apex. Heart conus $4\frac{1}{2} \times 5\frac{1}{2} \times 2$ cms. Epicard, endocard, and valves normal. Myocard slightly pale, and of slightly diminished consistence.

Diaphragm at fourth intercostal space on right, and at fifth on left side.

Peritoneum normal, without fluid. Abdominal organs normally situated.

Spleen : 10 × 5 × 2 cms. Capsule normal ; colour pale, with some dark patches, and a number of petechiae on the surface. Follicles prominent, light grey. Consistence normal.

Suprarenal capsules slightly hyperaemic, otherwise normal.

Right *kidney :* 6 × 4 × $1\frac{1}{2}$ cms. Capsule easily detached ; surface smooth, dark, shows capillary hyperaemia ; cortex grey ; pyramids slightly hyperaemic at bases, otherwise pale ; consistence normal. Left kidney : 6 × 4 × $2\frac{1}{2}$ cms., otherwise as the right. Renal pelves, ureters, and bladder normal ; bladder contains a small quantity of clear urine.

Genital organs normal.

Liver : 20 × 12 × 5 cms. Capsule normal ; colour of surface dark purple, with some yellow patches ; substance grey, likewise with yellow patches ; consistence normal.

Pancreas slightly enlarged, intensely hyperaemic, and of somewhat diminished consistence.

Tongue normal. *Tonsils* slightly enlarged, not hyperaemic. *Oesophagus* normal.

Stomach contains alimentary fluid ; mucosa normal.

Duodenum shows slight hyperaemia and swelling of follicles.

Jejunum normal. The lower part of the *ileum* shows considerable swelling of both solitary follicles and Peyer's patches ; the latter are hyperaemic. Appendix 7 cms., normal. Colon normal. In the lower part of the ileum two ascarides ; no other parasites.

Gall bladder and bile ducts normal ; the former organ contains greenish bile ; the latter are patent.

Mesenteric lymph nodules considerably enlarged, not hyperaemic.

Anatomical diagnoses : Poly-lymphadenitis, l.g. Hyperaemia meningum. Infarcti haemorrhagici pulmonum. Hyperaemia renum. Pancreatitis parenchymatosa acuta. Metamorphosis adiposa hepatis. Petechiae lienis.

Histological examination :—

Lymph nodules : slight hyperaemia. Moderate oedema of stroma.

Spleen : Marked oedema of stroma.

Liver : Intense fatty changes, moderate necrobiosis, both phenomena equally distributed throughout the lobules. The cells, when their limits are recognizable, are seen to contain a considerable number of large vacuoles (fat), but are in no case filled to distension with a single fat drop, nor do they contain large numbers of minute droplets. No hyperaemia. No inflammatory phenomena. Moderate oedema of stroma.

Kidney : Patches of intense hyperaemia ; epithelia of convoluted tubules in many places granular, and nuclei poorly staining or even absent, though the tubules are fairly well preserved. Glomeruli of normal appearances. Slight oedema of stroma. In osmic acid-preparations small fat droplets are seen in the epithelia of the convoluted tubules.

Brain : No hyperaemia.

In films made from the *spinal fluid* without centrifugalization no cells are seen. After centrifugalization very few cells which are mostly lymphocytes, also several polymorphonuclear leucocytes.

Microbiological examination :—

Nasgar tubes inoculated with spinal fluid ; one tube remains sterile and in another tube there is growth of (*a*) Gram-positive staphylococci, (*b*) Gram-negative diplococci forming chains, and changing maltose, but not glucose, galactose, saccharose and mannite. Heart blood : Nasgar tubes remain sterile ; on haemoglobin agar Gram-negative cocci develop similar to those in cultures of spinal fluid and showing marked formation of chains, but less marked diplococcal arrangement. No parasites are found on microscopical examination of spinal fluid, peripheral blood, heart blood, spleen and bone-marrow.

Case 49. M.A.W., 3 years 2 months, black, seen after death, with Dr. Earle, May Pen, who had seen the child on the previous day, when it was ill. February 19, 1913.

The child was quite well on February 17, and went to bed in the evening as usual, and slept well. On the 18th about 7 a.m. she was taken ill with convulsions which started suddenly ; there were contractions of arms and legs and rolling of the eyes ; no stiffness of neck observed. The attacks of convulsions followed each other rapidly. She was unconscious. She was seen by Dr. Earle at about 10 a.m. She was afterwards conscious and quiet until about 3 p.m., when convulsions again set in—about five occurred during the afternoon—and she again became unconscious and remained so until death, which took place at 9.45 p.m. She vomited about five times during the day, each vomiting occurring when a convulsion was about to set in.

Post-mortem examination, at 1 p.m., about fifteen hours after death. Body of well-nourished, absolutely healthy-looking child. No rigor. No jaundice. No cutaneous affection, and no parasites.

Spinal fluid flows freely by lumbar puncture, about 5 c.c. taken for examination ; it is perfectly clear.

The spinal venous plexus are extremely gorged ; dura and pia spinalis are moderately hyperaemic, pia especially in its lumbar portion ; no fibrinous exudation. Cord substance normal.

Dura mater cerebralis normal ; pia moderately hyperaemic, especially on posterior portion of the convex surface. No exudation. Lateral ventricles contain a few c.c. of clear fluid. Brain substance normal.

Cervical, axillary and *inguinal lymph nodules* moderately enlarged and slightly hyperaemic.

Diaphragm at fourth intercostal space on both sides.

Pleurae normal. *Lungs* contain a few small, haemorrhagic infarcts, and are slightly hyperaemic in postero-inferior portions; otherwise normal. The smaller as well as the larger bronchi, trachea, and larynx, contain a small amount of frothy mucus; otherwise perfectly normal.

The *pericardium* contains a small amount of clear fluid; heart conus $5 \times 6 \times 3$ cms. Pulmonary artery and aorta normal. Epicard, endocard, and valves normal. Myocard slightly pale, of somewhat pasty consistence.

Thymus: $9 \times 5 \times \frac{1}{2}$ cms. Appearance normal.

Thyroid body normal.

Peritoneum normal, without fluid. Position of abdominal organs normal; appendix inwards and downwards into the pelvis. Mesenteric lymph nodules moderately enlarged, not hyperaemic.

Spleen: $10 \times 1 \times 1\frac{1}{2}$ cms. Capsule normal; colour pale, with dark spots; follicles grey, slightly prominent; consistence normal.

Suprarenal capsules hyperaemic, of normal size.

Left *kidney:* $9 \times 4 \times 2$ cms. Capsule easily detached; surface pale, with somewhat dilated stellate veins; cortex grey with yellowish patches; pyramids slightly hyperaemic at their basis, otherwise normal. Right kidney, $8 \times 4 \times 1\frac{1}{2}$ cms., similar to the right one, but slightly more hyperaemic, and with more prominent yellowish patches.

Renal pelves hyperaemic, ureters and bladder normal; the bladder contains about 30 c.c. of clear urine.

Genital organs: hyperaemic, otherwise normal.

Liver: $20 \times 12 \times 5$ cms. Capsule normal; colour of surface distinctly yellowish with a few hyperaemic patches; tissue uniformly yellowish grey; structure normal; blood filling about normal; consistence somewhat diminished, pasty.

Pancreas: 11 cms. long; not hyperaemic; consistence considerably diminished.

The *stomach* contains a small quantity of alimentary mucus; the mucosa shows a few small patches of capillary hyperaemia.

Duodenum normal, as well as jejunum and ileum.

Colon shows a large number of black dots, corresponding to the centres of the follicles, which are not swollen.

The colon ascendens contains one Tricocephalus and two ankylostomes; no ankylostomes higher up in the intestine.

Bile ducts and *gall bladder* normal.

The whole surface of the *tongue* is covered with whitish fur.

Tonsils normal.

Oesophagus normal.

Anatomical diagnosis: Hyperaemia meningum. Poly-lymphadenitis. Hypostasis et infarcti haemorrhagici pulmonum. Metamorphosis adiposa myocardii et hepatis. Hyperaemia capsularum suprarenalium. Nephritis parenchymatosa acuta duplex l.g. Pancreatitis parenchymatosa acuta. Hyperaemia ventriculi l.g. Pigmentatio coli. Helminthiasis intestinalis.

Histological examination:—

Liver: Intense fatty metamorphosis, in some places most intense around portal vessels, in others equally distributed over all zones of the lobules. Capillary hyperaemia irregularly distributed. Slight necrobiotic changes.

Pancreas : No hyperaemia or cellular infiltration. No fatty change. The epithelia are as a rule normal, but in isolated places very poorly stained, the nuclei being almost invisible. Langerhans's islets well preserved and very definite.

Kidney : Some of the convoluted tubules show advanced granular and fatty metamorphosis, but in most the cells are fairly well preserved and the nuclei well stained. The glomeruli are considerably retracted.

Myocardium : slight fragmentation.

Spinal fluid : Numerous cells, mostly mononuclear ; only a few polymorphonuclear leucocytes.

Microbiological examination :—

Nasgar tubes inoculated with *spinal fluid* and *heart blood* remain sterile ; some sporulating Gram-positive bacteria develop in haemoglobin-agar tubes. In films from spinal fluid, after centrifugalisation, groups of diplococci are observed, also various bacteria.

Case 59. C.W., about 30 years, black, Barbican, seen after death, at Public Hospital Mortuary, with Dr. C. R. Edwards, of Half-Way Tree, on March 6, 1913.

Patient felt ill in the evening of March 5, vomited, had some whisky, felt better, woke up at about 1 a.m., on March 6, vomited again, had convulsions, became unconscious, and died about 6 a.m.

Post-mortem examination, on March 6, at about 2 p.m. Well-nourished, healthy-looking body. No skin diseases, nor parasites. No jaundice.

Lumbar puncture gives an abundant quantity of faintly cloudy fluid ; about 60 c.c. are collected, but the fluid is still flowing when the needle is withdrawn. Spinal dura, pia, and cord normal. Cerebral dura, pia, likewise normal, as well as the brain ; lateral ventricles not distended.

Hypophysis cerebri : 13 × 6 × 3 mms., slightly hyperaemic.

Subcutaneous fat intensely yellow, orange, not icteric, but reminding of the condition described from German Africa, as due to the ingestion of a certain kind of vegetable oil. Visceral fat of the same colour.

Cervical, axillary, and *inguinal lymph nodules* considerably enlarged and hyperaemic, the axillary mostly so.

Diaphragm at the fourth intercostal space on the right side, at the fifth on the left.

Both *pleurae* show total, fibrous adhesions. The *lungs* show slight hyperaemia and oedema of the postero-inferior portions. The right lung contains in its superior and inferior lobes several fibro-calcareous noduli. The smaller bronchi of both lungs show moderate hyperaemia and swelling of their mucosae, and some mucous secretion. Larger bronchi, trachea, and larynx normal.

Bronchial lymph nodules slightly enlarged and anthracotic.

Thyroid body normal.

Pericardium shows total, fibrous adhesions. Heart conus, 9 × 10 × 5 cms. Left ventricle fairly well contracted, forms apex. Pericardial fat shows several ecchymoses on anterior surface of right ventricle. The mitral valve shows several thickened, yellow patches on the anterior flap, and some nodular fibrous thickening of free edge. There is no stricture nor insufficience of the valve, and the other valves and endocard are normal. Myocard 1 cm. max. in left, 3 mm. in right ventricle ; it shows yellowish stripes and small patches ; consistence somewhat diminished. Pulmonary artery normal. The aorta shows extensive and intensely yellow patches of different sizes, some with superficial loss of substance, others with calcareous plaques ; the patches make their appearance immediately

over the aortic valve, are most prominent in the arch, and are continued far down into the abdominal portion.

The diaphragm shows over right lobe of liver a hard fibrous nodule, of about the size of a small walnut, and close to the nodule a small, serous cyst.

Peritoneum normal. Abdominal organs normally situated.

Spleen: 11 × 7 × 2 cms. Capsule adherent; tissue dark purple, with prominent grey follicles; substance soft.

Suprarenal capsules normal.

Left *kidney:* 11 × 6 × 3 cms. Capsule easily detached; surface smooth, pale grey, with patches of slight capillary hyperaemia; cortex pale grey, hyperaemic stripes and somewhat prominent glomeruli; columnae Bertini somewhat bulging forwards on the cut surface, which is pale yellowish grey; pyramids pale; consistence considerably diminished. The right kidney shows the same size and characteristics as the left one. Renal pelves, ureters, and bladder normal; the bladder contains about 500 c.c. of clear, pale urine.

Ovaries contain several serous cysts, no corpora lutea, and both ligg. lata contain also serous, multilocular cysts. Right tuba normal; left contains serous liquid in its considerably distended fimbriate extremity.

Corpus uteri: 9 cms; collum 5 cms., transverse diameter of corpus 8 cms. The perimetrium is normal, except for a small serous cyst on left surface. The endometrium cervicis appears normal, the endometrium corporis is slightly swollen and covered with muco-purulent secretion. The posterior wall of the corpus contains an almost globular mass, 6 cms. in diameter; consistence soft, elastic. Section shows a brain-like structure with some fatty masses. The anterior wall contains a small, submucous nodule, of similar aspect and consistence as the larger one.

Liver: 27 × 14 × 6 cms. Capsule normal; surface spotted, purple and dark yellow with smaller and larger hyperaemic patches, the whole aspect similar to that of nutmeg liver; consistence diminished, somewhat friable.

Bile ducts normal, patent; gall bladder normal, contains greenish bile.

Pancreas of normal size, not hyperaemic, of slightly diminished consistence.

Tongue coated with thick, white fur.

Tonsils normal.

Oesophagus normal.

Stomach contains alimentary fluid; the mucosa is hyperaemic in the neighbourhood of the lesser curvature, and in the cardiac portion, where several patches of petechiae are seen.

Duodenum slightly hyperaemic. Jejunum and ileum normal, except for a few patches of capillary hyperaemia. Appendix normal.

Colon shows patches of capillary hyperaemia.

No intestinal parasites.

Mesenteric lymph nodules slightly enlarged, not hyperaemic.

Urine: contains no albumen, no sugar and no casts.

Anatomical diagnoses: Pigmentatio telae adiposae. Poly-lymphadenitis acuta. Sequelae pleuritidis duplicis. Noduli calcareo-fibrosi (tuberculosi) pulmonis dextri. Bronchitis capillaris l.g. Sequelae pericarditidis. Endocarditis mitralis chronica fibrosa. Metamorphosis adiposa myocardii. Aortitis atheromatosa et calcarea. Nodulus fibrosus diaphragmatis. Splenitis acuta. Perisplenitis chronica. Nephritis acuta parenchymatosa duplex. Cystes ovarii utriusque. Hydro salpinx dextra. Cystis parva perimetrii. Tumor intramuralis uteri (Sarkoma) Tumor (metastaticus) endometrii. Metamorphosis adiposa et hyperaemia hepatis. Hyperaemia et petechiae mucosae ventriculi.

Histological examination :—

Lungs : Large fat drops are present in nearly all epithelial cells.

Pancreas : Most epithelial cells contain small fat granules. Extensive necrobiosis occurs, irregularly distributed. Langerhans's islets being affected as well as the epithelium proper ; there is considerable hyperaemia which is likewise irregularly distributed, in some cases apparently limited to Langerhans's islets ; there is also some infiltrating haemorrhage.

Kidney : Sections show considerable increase of connective tissue, especially in the pyramidal substance ; the arterial walls are fibrous and increased in thickness, as well as Bowman's capsules ; there are patches of hyperaemia and in some places interstitial haemorrhages. The epithelia of the convoluted tubules show in some places granular protoplasm, but contain no vacuoles. Their nuclei are in some places poorly stained or absent, but as a rule they are well preserved. In osmic acid specimens small fat granules are seen in nearly all epithelial cells in the convoluted tubules and also in the lining epithelia of glomeruli and Bowman's capsules.

Numerous ' mastzellen ' are seen in the interstitial tissues.

Uterine tumour : The tumour shows the structure of a fibro-myoma, but of somewhat atypical characters, being very rich in cells which show considerable polymorphism and often large ovoid or irregularly-shaped nuclei (sarcoma).

Microbiological examination :—

Cultures on nasgar from *spinal fluid* remain sterile for several days, later some Gram-positive staphylococci develop in one tube and diptheroid bacilli in another, Gram-positive staphylococci develop in cultures of *heart blood* (nasgar).

No parasites in smears from *heart blood.*

The following cases are similiar to the preceding ones and are therefore only given in abstract.

Case 37. G.B., 16 months, black, sister to E.B. case, Rock near Four Paths, with Dr. E. R. C. Earle, May Pen.

Sudden onset of vomiting at 10 a.m., on February 4, 1913. The patient is said to have had retraction of head, but no convulsions. Slight strabismus. Temperature normal ; pulse 140, small, slightly irregular. Death February 5 at 11 p.m.

Post-mortem examination :—

Spleen : Numerous subcapsular petechiae.

Kidneys : Pyramidal bases intensely hyperaemic. Consistence considerably diminished. The left kidney shows pronounced superficial hyperaemia and a few minute haemorrhages on the surface.

Liver : Surface pale, yellowish, and pronouncedly yellow in some parts ; in other places considerable hyperaemia. The consistence of the yellow portions is soft, whilst it is about normal in the hyperaemic patches.

The *stomach* contains a considerable amount of dark, grey, alimentary fluid, with no evidence of haemorrhage. Mucous membrane pale, of normal appearance. The duodenum shows several hyperaemic patches. The jejunum and ileum present likewise some hyperaemic patches, but the hyperaemia is much less pronounced than in the duodenal patches.

A few ankylostomes and a few ascarides are found in the upper portion of the jejunum ; no tricocephalus.

Anatomical diagnoses : Hyperaemia piae matris cerebralis et oedema incipiens. Lympho-adenitis universalis. Nephritis acuta duplex. Metamorphosis adiposa

hepatis m.g. Pancreatitis parenchymatosa acuta. Hypostasis pulmonum. Hyperaemia et ecchymoses pericardii. Metamorphosis adiposa myocardii. Helminthiasis intestinalis.

Microbiological examination :—

Spinal fluid : Nasgar tubes : Gram-negative diplococci, which decompose both glucose, maltose, galactose, lactose and saccharose after five days.

Case 42. C.W., 2½ years, black, Spanish Town Road, with Dr. C. R. Edwards, Half-way Tree.

The previously healthy child woke up at 4 a.m., on February 14, 1913, and vomited a small quantity of frothy mucus ; convulsions set in, and coma, from which she never recovered ; she died at about 8 a.m., at the D.M.O.'s (Dr. Edwards's) office, to which she had been taken just previously ; Dr. E. obtained blood smears, spinal fluid, and urine before death.

Post-mortem examination, at 3 p.m., on February 14, seven hours after death.

Spinal meninges and cord normal, not hyperaemic. Cerebral dura slightly hyperaemic, but pia of perfectly normal appearance. Brain normal.

Right *pleura* normal ; left pleura shows slight, but extensive adhesions and numerous petechiae. Both lungs show a few small haemorrhagic infarcts, but are otherwise normal, without hypostasis. Mucosae of smaller and larger bronchi, and of trachea, and larynx, normal.

Both *suprarenal capsules* slightly enlarged, and both, especially the left, show haemorrhagic infiltration.

Kidneys : Capsule easily detached ; surface smooth, with slight capillary hyperaemia ; cortex normal ; pyramids pale.

The *stomach* contains a small quantity of bile-stained mucus ; its mucosa is perfectly normal, as is also that of the duodenum.

The *liver* measures 23 × 14 × 5 cms. Capsule normal ; colour dark purple on the surface ; the tissue is grey, with slight increase of the portal connective tissue ; consistence on the whole slightly increased, but in some places friable.

Pancreas slightly hyperaemic, of normal consistence.

The *lymph nodules in the hepatic hilus* are very considerably enlarged, of greenish-grey colour, and of soft elastic consistence.

Urine contains no albumin, and no casts.

Anatomical diagnoses : Poly-lymphadenitis acuta. Hyperaemia durae matris cerebralis l.g. Sequelae pleuritidis sinistrae. Petechiae pleurae sinistrae. Infarcti haemorrhagici pulmonis utriusque. Splenitis chronica. Epinephritis haemorrhagica duplex. Enteritis follicularis l.g. Helminthiasis intestinalis (one *Ascaris lumbricoides*).

Histological examination :—

Liver : Advanced fatty change, practically all cells being filled to distension with moderately-sized fat drops. Some irregularly distributed capillary hyperaemia and very considerable microcellular infiltration, penetrating from the periportal tissues into the parenchyma.

Bacteriological examination :—

Spinal fluid : In nasgar tubes growth of Gram-negative diplococci which decompose glucose, maltose and galactose after two days, and lactose and saccharose after four days.

Case 52. J.E., 6 years, black, seen, after death, with Dr. C. R. Edwards, February 24, 1913.

The patient is a brother to Elisabeth E. (case 51), and was taken ill suddenly about 8 a.m., on February 22, shortly after his sister; in fact the family ascribed his illness to his having been frightened by seeing the sister's convulsions. He had several convulsions, but no vomiting. He died comatose, at about 2 a.m., on February 23. He was seen by Dr. Croswell.

Duration of illness: eighteen hours.

Post-mortem examination:—

Axillary and cervical nodules considerably, inguinal slightly, enlarged and hyperaemic.

The *stomach* contains alimentary fluid; mucosa normal, not hyperaemic.

Anatomical diagnoses: Hyperaemia meningum. Hyperaemia glandulae suprarenalis sin. et hypophysis cerebri. Hypostasis pulmonum. Splenitis acuta. Nephritis parenchymatosa acuta duplex l.g. Metamorphosis adiposa myocardii, hepatis, et pancreatis. Pigmentatio coli. Cadaverositas universalis.

The *histological examination* confirmed the anatomical diagnoses.

Bacteriological examination: No growth in nasgar tubes; in haemoglobin-agar tubes growth of Gram-negative bacteria.

Case 56. R.W., infant, black, seen with Dr. Gifford, at the Public Mortuary, Kingston, on March 3, 1913.

Post-mortem examination:—

Stomach shows considerable hyperaemia and a few petechiae.

Anatomical diagnoses: Hyperaemia piae matris cerebralis. Poly-lymphadenitis. Hypostasis pulmonum. Metamorphosis adiposa myocardii l.g., pancreatis, et hepatis l.g. Nephritis parenchymatosa acuta duplex incipiens. Gastritis ecchymotica l.g. Lymphadenitis mesenterialis. Helminthiasis intestinalis (*Ascaris lumbricoides*).

The *histological examination* confirmed the anatomical diagnoses except that the liver showed a rather intense fatty metamorphosis and some necrobiotic changes. The pancreas showed, in addition to fatty change, some localized hyperaemia, interstitial haemorrhages and fibrinous exudation; also slight oedema of stroma; the islets of Langerhans were of normal aspect.

Microbiological examination:—

No growth in nasgar tubes inoculated with *spinal fluid.*

Case 60. I.R., 4½ years, black, Four Roads, seen, after death, on March 7, 1913, at Kingston Hospital Mortuary, with Dr. C. R. Edwards.

Previous health good. On March 6, in the evening, 'she complained of abdominal pain, and later on she vomited. She went to sleep. Next morning she woke up early, screaming; she had convulsions, became comatose, and died.'

Post-mortem examination:—

Lumbar puncture gives clear fluid, with admixture of blood. Intra-spinal vessels intensely congested. Spinal dura and pia slightly hyperaemic. Cerebral dura normal, pia slightly hyperaemic, especially over cerebellum. Brain and cord normal.

Cervical, inguinal, and axillary lymph nodules enlarged and hyperaemic, especially the latter.

Stomach normal, contains alimentary fluid. Duodenum shows considerable follicular swelling, and the ileum shows slight swelling of solitary follicles and Peyer's patches in its lower part; the colon likewise shows follicular swelling; appendix 6 cms., normal.

Anatomical diagnoses : Hyperaemia meningum l.g. Poly-lymphadenitis acuta. Hypostasis pulmonum. Splenitis acuta. Nephritis acuta. Enteritis follicularis. Metamorphosis adiposa hepatis. Petechiae pericardii visceralis.

Histological examination of liver and kidneys confirmed the anatomical diagnoses.

Bacteriological examination :—

No growth in nasgar tubes (*spinal fluid* and *heart blood*).

Case 61. C.W., 12 years, black, Whitehall, seen, after death, with Dr. C. R. Edwards, at Kingston Public Hospital Mortuary, 8-III-i.

Was taken ill on March 6, with severe abdominal pain. No rise of temperature. No vomiting. No convulsions. Died March 7, at 4 a.m.

Anatomical diagnoses : Hyperaemia meningum. Hypostasis pulmonum. Poly lymphadenitis acuta. Hyperaemia et petechiae mucosae ventriculi. Helmin thiasis intestinalis. Cadaverositas universalis.

Bacteriological examination : Nasgar tubes remain sterile (spinal fluid).

Group B consists of cases in which 'meningococci' were found in the cerebro-spinal fluid, whilst definite inflammatory lesions of the meninges were absent, although a more or less marked hyperaemia was observed. Isolated cases of this nature, observed during an epidemic of cerebro-spinal meningitis, would be accepted under this diagnosis without much discussion. The peculiar circumstances in our case, and more especially the close resemblance of these cases to those of Group A, make it, however, necessary to consider very seriously the possibility that the diplococcus infection might be a secondary one. This question is one of the most essential of the whole problem and will be discussed together with the general aspects of 'vomiting sickness.' This group includes the Cases 1, 7, 8, 12, 20, 30, 33, 43, 44, 50, 55 and 57, a total of 12 cases.

As in the case of Group A, several cases are described in full, as types, and others only abstracted. Some of the cases are almost identical with those given as types in Group A. The post-mortem appearances of the meninges varied considerably in this group also, from slight hyperaemia to intense hyperaemia accompanied by oedema; in one case there was no hyperaemia at all and the meninges appeared perfectly normal. Similarly, the gastric mucosa showed in some cases normal conditions, in others hyperaemia, and in others again, both hyperaemia and petechiae. The conditions found in the stomach in Case 21 are particularly interesting.

Case 7. J.J.G., about 8 years, black, Chandler's Pen. Autopsy at 3.30-5 p.m., about twenty-six hours after death, January 18, 1913.

The child was healthy until the foregoing day—January 17—at about 4 a.m. when he was taken ill with vomiting, of watery fluid and food. He was conscious to begin with, but soon became unconscious, had twitching of the limbs, vomited a little again, and became gradually weaker, and died at 2 p.m. (he was not seen by medical man until after his death).

Post-mortem examination (with Dr. Earle). Fairly well-nourished child. Rigor present. Slight jaundice of sclerae.

Spinal puncture : about 20 c.c. of clear fluid.

Dura mater normal. *Pia mater cerebralis* shows slight diffuse hyperaemia.

Brain and *spinal cord* macroscopically normal.

Hypophysis cerebri appears likewise normal.

Tongue coated.

The *tonsils* are slightly enlarged, but show no evidence of acute inflammation.

Larynx, *trachea*, and *bronchi* contain bloody, frothy mucus, otherwise normal.

Bronchial lymph nodules enlarged and anthracotic.

No adhesions but a few c.c. of clear fluid in each of the pleural cavities.

The *lungs* show slight hyperaemia of the lower lobules, and some oedema of right superior lobe, but no infiltrations. No tubercular phenomena in lungs or lymph nodules.

Thyroid body normal.

About 10 c.c. of clear fluid in the pericardial cavity.

Heart : 5 × 6 × 2½ cms. No pericardial haemorrhages. Myocardium slightly pale, shows no evidence of fatty metamorphosis.

No liquid in abdominal cavity. Peritoneum appears normal.

Spleen : 11 × 6 × 2½ cms. Capsule smooth, transparent. Colour dark.

Suprarenal capsules normal.

Left *kidney*, 8 × 4 × 1½ cms. Capsule easily detached. No superficial hyperaemia, but on section hyperaemia of pyramidal bases, which stand out very dark on the pale cortical substance. Right kidney as left.

Renal pelves and *ureters* normal.

The *bladder* presents a normal mucosa, contains a considerable amount of clear urine.

Liver : 21 × 11 × 5 cms. Capsule smooth ; transparent. No haemorrhages. Consistence normal, homogeneous. Lobuli very prominent, of an intense yellowish red colour.

The *pancreas* is somewhat enlarged, and is the seat of considerable hyperaemia. Substance extremely soft.

Oesophagus normal.

The *stomach* contains alimentary fluid. The mucosa is hyperaemic, especially in the vicinity of the lesser curvature. In this region numerous minute petechiae.

The *duodenum* shows very slight hyperaemia, but no haemorrhages. Jejunum, slight hyperaemia which becomes less pronounced lower down and is not at all observed in the ileum. No enlargement of solitary follicles nor of Peyer's patches. Four ascarides in the small intestine, and a few tricocephali in coecum. No ankylostomes.

Bile ducts and *gall-bladder* show normal mucosa ; the bladder contains dark brownish bile.

The large bowel and *vermiform appendix* show normal conditions.

Anatomical diagnoses : Hyperaemia piae matris cerebralis l.g. Tonsillitis chronica. Anthracosis lympho-bronchialis. Nephritis parenchymatosa subacuta.

Hyperaemia et petechiae mucosae ventriculi. Helminthiasis intestinalis (*Ascaris lumbricoides*, *Tricocephalus trichiurus*).

Histological examination :—

Mesenteric lymph nodules : oedema of stroma.

Liver : Marked fatty change, evenly distributed throughout the lobules. Hyaline and oedematous thickening of the stroma, corresponding both to portal and to central veins.

Pancreas : Interstitial oedema.

Spleen : Oedema of coarse trabeculae.

Kidney : No oedema, but slight increase of stroma.

Microbiological examination :—

Nasgar tubes inoculated with spinal fluid show growth of Gram-negative diplococci which change glucose and maltose but not saccharose, lactose, galactose and mannite.

Case 12. A.M., 3 years, black, at 'Four Roads,' St. Andrew (seen with Dr. L. O. Croswell).

The girl has been subject to colds in the head, but has otherwise been healthy. She has never passed any worms, according to her family. In the morning of January 21, 1913, she appeared slightly unwell, and at noon-time she started vomiting clear fluid and continued vomiting during the afternoon. She had no febrile symptoms, but at 5 p.m. convulsions set in. Dr. Croswell saw the patient at 5.30 p.m. ; she was then comatose and limp, showing general muscular relaxation. There was no rigidity, no Kernig's symptom, and no abdominal retraction. Four or five seizures were observed, lasting for about half a minute each time, and consisting in tonic, rather than clonic, contractions. There was seen froth at the mouth, and a sort of hiccough, but no vomiting. She died at 6.15 p.m., apparently from respiratory failure ; the pulse could be felt, and the heart went on beating for some time after breathing had stopped altogether.

The pulse was rather weak during the whole of the time during which the child was under observation. Adrenalin was given subcutaneously, as a stimulant, by Dr. Croswell.

Two years ago a brother of the deceased (then about 11 years old) had similar symptoms, but recovered under stimulating treatment. This boy was taken ill at a time when an epidemic outbreak of vomiting sickness reigned at 'Four Roads.'

Post-mortem examination, at 10 a.m., on January 22, with Dr. Edwards. Body well nourished. No rigor. No jaundice.

Spinal fluid is taken by lumbar puncture ; it appears clear, but is mixed with blood.

Tongue clean. *Tonsils* normal. *Larynx*, *trachea*, and *bronchi* without pathological changes, and empty, except the secondary bronchi which contain a small quantity of frothy mucus.

No adhesions, and no fluid in the pleural cavities.

Lungs slightly hyperaemic in postero-inferior parts. No oedema, and no infiltrations. No infarcts.

Bronchial lymph nodules not enlarged, of pinkish colour.

No anthracosis, and no tubercular phenomena in lungs and bronchial lymph nodules.

Heart : $3\frac{1}{2} \times 4\frac{1}{2} \times 2$ cms. About 5 c.c. of clear fluid in pericardium. Pulmonary artery free. Left ventricle well contracted. Myocardium pale,

greyish, without haemorrhages and yellowish patches. Consistence slightly diminished.

Thyroid body and *Thymus* macroscopically normal.

Peritoneum of normal aspect. Position of abdominal viscera normal.

Spleen : 6 × 3½ × 1 cms. Capsule smooth, transparent. Colour dark, with pale, prominent follicles. Consistence normal.

Mesenteric lymph nodules considerably enlarged, of pinkish colour, with slightly prominent vessels. Consistence normal.

Suprarenal bodies macroscopically normal.

Left *kidney :* 6 × 3½ × 1½ cms. Capsule easily detached. Surface smooth, stellate veins hardly visible. Cortex pale, especially in proximal parts. Small yellowish patches irregularly distributed in cortical substance. Pyramidal bases slightly hyperaemic, apices pale. No haemorrhages. Consistence slightly diminished. Right kidney : Same dimensions and aspect as left, only slightly paler.

Renal pelves, *ureters*, and *bladder* normal, the latter containing a few c.c. of pale urine.

Oesophagus normal.

The *stomach* contains a small quantity of alimentary fluid. The mucosa is slightly swollen and shows diffuse hyperaemia, mostly pronounced in the vicinity of the lesser curvature. In this portion of the mucosa there are a few groups of minute petechiae.

Duodenum : Slight hyperaemia.

Jejunum, *ileum*, and *large bowel* pale, without ulcers and haemorrhage. Solitary follicles enlarged in ileum, and jejunum, and Peyer's patches enlarged and hyperaemic.

Liver : 17 × 11 × 5 cms. Capsule smooth, transparent. Colour irregular, pinkish, with yellowish patches of different sizes, and a few hyperaemic patches. Substance homogeneously yellowish grey ; the small blood vessels hardly visible. Consistence slightly diminished, not friable.

Pancreas : 10 cms. long. Tissue slightly hyperaemic, and of considerably diminished consistence.

Bile ducts and *gall bladder :* Mucosa normal. Considerable amount of dark greenish yellow bile in bladder.

Genital organs normal, except that both ovaries contain small serous cysts.

Meninges : Dura mater normal. Pia mater spinalis normal ; pia cerebralis slightly hyperaemic, in particular on the convex surface. No oedema and no inflammatory exudations. Lateral ventricles not enlarged, contain a very small quantity of clear fluid.

Brain and *spinal cord* macroscopically normal.

Anatomical diagnoses : Hyperaemia piae matris cerebri. Hypostasis pulmonum levi gradu. Anaemia et metamorphosis parenchymatosa myocardii. Hyperaemia oedema levi gradu et petechiae minimae mucosae ventriculi. Enteritis follicularis. Anaemia et metamorphosis adiposa hepatis. Anaemia renum (et metamorphosis adiposa ?). Pancreatitis parenchymatosa acuta.

Histological examination :—

Liver : Intense fatty metamorphosis and very slightly marked necrobiotic phenomena. Marked increase of connective tissue, especially around portal vessels, but also penetrating into the lobules, separating small groups of cells from each other ; considerable microcellular infiltration, especially in the portal stroma.

Spleen : Intense diffuse hyperaemia ; follicles show necrobiotic changes.

Brain : No hyperaemia, no perivascular cell-infiltration.

Kidney : Marked granular appearance and moderate vacuolization (fat) of epithelia of convoluted tubules ; marked karyolysis in the same class of epithelia ; glomeruli considerably retracted. Marked increase of stroma, especially in pyramidal portions, slight microcellular infiltration.

Pancreas : Patches of beginning necrobiosis ; Langerhans' islets somewhat prominent.

Microbiological examination :—

Heart blood, nasgar tubes remain sterile for four days. Sanguinolent spinal fluid : Gram-negative diplococci (in the original culture the results of Gram and Claudius staining were somewhat irregular, in subculture the results were always negative), which change glucose, maltose, and galactose but not lactose, saccharose, and mannite. Spleen smears show small numbers of plump bacilli and a few chains of diplococci.

Case 20. T.J., 12 years, coolie (Hindoo), May River, St. Mary, seen on January 31, 1913, with Dr. Joslen, Annotto Bay. Was living in a dirty hut, with his father, mother and two brothers, all in a half-starved condition. The father was ill with stomatitis, vomiting and dysenteriform diarrhoea, and the mother was dying from phthisis, with acute pneumonia. The brothers were in good health. According to later information, the father recovered but the mother died.

Water supply from river ; no latrines ; house on the side of a steep hill, drainage consequently good.

Deceased retired to bed on the evening of January 30, apparently well. Next morning he was found dead in his bed. (Clinical notes by Dr. Joslen).

Post-mortem examination, on January 31, at about 6 p.m. Poorly-nourished body. Rigor present. No jaundice. No cutaneous affections, but numerous insect bites and scratches on lower extremities.

Spinal puncture gives no fluid. Dura normal. Slight diffuse hyperaemia of pia spinalis and cerebralis, mostly pronounced over cerebellum. No oedema, no exudation. Lateral ventricles not dilated. Brain and cord normal.

Pleurae normal. Lungs slightly anthracotic, otherwise normal.

Bronchial lymph nodules normal.

Larynx, *trachea*, and *bronchi* are hyperaemic and contain frothy fluid.

Thyroid body pale, otherwise normal.

Pericardium normal. Endocardium and valves normal ; myocardium likewise, as well as pulmonary artery and aorta.

Peritoneum normal. Abdominal organs normally situated.

Spleen : 16 × 10 × 3 cms. Capsule normal ; colour dark greyish red ; consistence semi-fluid. Tissue uniform ; follicles greyish, not prominent.

Suprarenal capsules normal.

Left *kidney :* 8 × 5 × 1½ cms. Capsule easily detached ; surface smooth, reddish with large yellowish patches ; stellate veins not distended ; tissue uniformly greyish red, with yellow patches in cortex. Consistence slightly diminished ; the right kidney presents the same characters as the left.

Liver : 20 × 15 × 6 cms. Capsule normal ; colour reddish grey, with yellow patches, and on the right lobe a number of minute haemorrhages ; consistence normal.

Pancreas hyperaemic, soft.

Tongue heavily coated. Tonsils without signs of acute inflammation.

The *stomach* shows on the central portion of the anterior wall eight dark, almost black, blood clots embedded in the mucous membrane ; when removed a red spot is left, but no actual opening of a vessel is to be seen. No hyperaemia of the mucosa and no petechiae.

Duodenum normal. The small intestine contains in many parts, especially in the middle part of the jejunum, numerous small, half-digested blood coagula, which are freely movable ; no haemorrhages in the mucosa, no ulcers, no follicular swelling and no hyperaemia. Appendix and large bowel normal.

Anatomical diagnoses : Hyperaemia piae matris. Splenitis acuta. Pancreatitis acuta parenchymatosa. Nephritis parenchymatosa acuta duplex l.g. Metamorphosis adiposa hepatis l.g. Haemorrhagiae ventriculi.

Histological examination :—

Spleen : Diffuse hyperaemia.

Liver : Localized hyperaemia. Cell infiltration of portal connective tissue. Moderate fatty change. No oedema of stroma.

Pancreas : Oedema of stroma. Necrobiotic changes of parenchyma.

Kidney : Slight oedema of stroma. Advanced necrobiotic changes of epithelia in convoluted tubules.

Microbiological examination :—

Spinal fluid, nasgar : Gram-negative diplococci which change glucose and maltose, but not lactose, galactose, saccharose, and mannite.

Case 44. E.P., 2 years, black, Kings Gate, seen, after death, with Dr. C. R. Edwards, Half-way Tree.

Previous health good. Sudden onset of vomiting, followed by convulsions, death in 3-4 hours.

Post-mortem examination, February 15, 1913. Body fairly well nourished ; rigor present ; no jaundice ; on the buttocks and lower extremities numerous scars as from scratching and small furunculi. Lice in hair, no other cutaneous parasites.

Lumbar puncture gives about 3 c.c. of clear fluid. Dura and pia spinalis show slight hyperaemia. Dura cerebralis normal ; pia shows slight hyperaemia of convex surface ; no exudation. Brain and cord normal. No tubercular affection.

Inguinal, axillary and *cervical lymph nodules* moderately swollen ; some of the inguinal and cervical nodules hyperaemic.

Diaphragm at fourth intercostal space on the right side, at the fifth on the left.

Pleurae without adhesions, contain no fluid ; the right pleura on the inferior lobe shows a number of miliary tubercles.

Lungs slightly hyperaemic, especially in the postero-inferior portions ; left lung otherwise normal. The right lung shows in its middle and inferior lobes numerous peribronchial tubercular nodules.

Bronchial lymph nodules on the right side are considerably enlarged and show caseous changes.

The smaller bronchi of the right lung show in many places caseous infiltration ; those of the left lung, as well as the larger bronchi, trachea and larynx show hyperaemic swelling of the mucosa and considerable mucous secretion.

Thymus : $9 \times 5 \times \frac{1}{2}$ cms ; appearance and consistence normal.

Thyroid body normal.

Pericardium contains a few drops of clear fluid. Pulmonary artery and aorta normal. Epicard, endocard and valves normal. Heart conus $5 \times 5 \times 2\frac{1}{2}$ cms. ; left ventricle fairly well contracted ; myocard slightly pale, of normal consistence.

Peritoneum normal, without fluid ; abdominal organs normally situated.

Tongue covered with white fur, on its whole surface. Tonsils slightly enlarged ; no signs of acute inflammation, no haemorrhages and no tubercular phenomena.

Oesophagus normal.

Stomach contains alimentary fluid ; mucosa shows follicular swelling, is otherwise normal.

Duodenum shows intense follicular swelling. Jejunum, ileum and appendix normal. Colon shows slight follicular swelling and punctate pigmentation corresponding to the folliculi.

The *bile ducts* are patent; their mucosa is normal, as well as that of the gall bladder, which contains a moderate quantity of greenish bile.

Liver: 19 × 12 × 5 cms. Capsule normal; colour dark purple; tissue shows a few yellowish patches, is otherwise normal.

Pancreas of normal size and colour, but of diminished consistence.

Spleen: 9 × 4 × 1½ cms. Capsule normal; colour normal; consistence soft; the tissue contains a number of miliary tubercles.

Suprarenal capsules normal.

Left *kidney:* 7 × 3 × 2½ cms. Capsule easily detached; surface smooth, pale, with considerable distension of stellate veins; cortex pale grey with yellowish patches; pyramids slightly darker, but not hyperaemic; consistence diminished. A few miliary tubercles in the tissue. Right kidney as the left, except that it does not contain any tubercles. Mucosa of renal pelves, ureters and bladder normal.

Genital organs normal.

Urine: faintly acid, contains a small quantity of albumen and a few granular casts.

Histological examination:—

Cervical medulla: Moderate hyperaemia.

Spleen: Diffuse hyperaemia; thickening of arterial walls. No histological tuberculosis.

Myocardium: Moderate fatty change.

Lungs: Caseous and miliary tuberculosis.

Bronchial lymph nodules: Hyperaemia; caseous and miliary tuberculosis.

Liver: Fatty change, in some parts moderate, in others intense.

Pancreas: Localized hyperaemia, partial necrobiosis, prominent Langerhans' islets.

Kidney: Diffuse hyperaemia, granulated protoplasma of epithelia in some convoluted tubules and nuclei sometimes poorly stained.

Stomach: The epithelial cells are as a rule fairly well preserved; some of them considerably swollen (hypersecretion ?). Glands are seen beneath the ordinary level of the mucosa completely embedded in connective tissue, but apparently not penetrating the muscularis mucosae which has been displaced, following as a continuous layer underneath the glands. Lymphoid tissue is scarce; there is moderate hyperaemia.

Case 50. J.M., 3 years, black, Old Hope Road, St. Andrew, seen at Kingston Public Hospital, with Dr. Thomson, February 21, 1913.

Admitted, at 12 noon, on February 21, with a history of convulsions of sudden onset, from early in the morning on the same day.

She is unconscious; there is no rigidity of the limbs; head not retracted; muscles of neck somewhat rigid; Kernig's sign not obtained. Knee-jerks absent. Pupils somewhat dilated, no reaction to light. A fresh, small burn is seen on the left wrist (hot bottles had been applied at home).

Temperature 98·4. Breathing laboured. Moist râles over front of chest. Heart's action very rapid and irregular. Abdomen not distended, nor retracted. Bowels act after enema. No vomiting.

The temperature went down to 98·2 later on; the child had several attacks of convulsions, during which the extremities were in a state of tonic contraction, and

were moved slowly to and fro; no clonic movements were observed. One attack was observed for at least ten minutes, and lasted probably longer.

The child died at 5.30 p.m.

Post-mortem examination: February 22, at 10.30 a.m., seventeen hours after death. Body fairly well nourished. Slight rigor. No jaundice. No skin affection.

Cervical, axillary and inguinal lymph nodules considerably enlarged and somewhat hyperaemic.

Spinal fluid not obtained, as skull had been opened by assistant.

Dura cerebralis and *spinalis* normal; pia moderately hyperaemic on convex surface of brain; spinal pia not hyperaemic, but shows dark pigmentation. Cord and brain substance normal; the lateral ventricles contain a few drops of serous fluid, from which culture is made.

Hypophysis cerebri normal.

Pleurae normal. Both lungs show slight diffuse hyperaemia, and, on the cut surface, mucous secretion escaping from the capillary bronchi. The smaller and larger bronchi, and trachea, and larynx are considerably hyperaemic, and contain mucous secretion.

Bronchial lymph nodules moderately enlarged and hyperaemic. No tubercular changes.

The *pericardium* contains a few c.c. of clear fluid. Heart conus $4 \times 5 \times 2\frac{1}{2}$ cms.; left ventricle somewhat flabby; epicard, endocard and valves normal. Myocard pale, with yellowish patches, of diminished consistence. Pulmonary artery and aorta normal.

Thymus: $9 \times 3 \times \frac{1}{2}$ cms.; of normal appearance.

Thyroid body normal.

Peritoneum normal, without fluid. Abdominal organs normally situated. Appendix in internal position, between the small intestines.

Diaphragm at third intercostal space on right side, at fourth on left.

Mesenteric lymph nodules considerably enlarged, but not hyperaemic.

Spleen: $7 \times 4 \times 1$ cms. Capsule normal; numerous subcapsular and intraparenchymatous ecchymoses; tissue pale; follicles not prominent; consistence slightly diminished.

Left *suprarenal capsule* hyperaemic, of normal size; right normal.

Left *kidney:* $7 \times 4\frac{1}{2} \times 2\frac{1}{2}$ cms. Capsule easily detached; surface smooth; pale, with patches of capillary hyperaemia; cortex pale yellowish grey and columnae Bertini even more pronouncedly so; pyramidal bases intensely hyperaemic. Consistence considerably diminished.

Renal pelves, *ureters* and *bladder* normal; the bladder contains a small quantity of pale urine.

Tongue covered on the whole surface with thick, whitish fur.

Tonsils enlarged, without signs of acute inflammation.

Pharynx hyperaemic.

Oesophagus normal.

The *stomach* contains alimentary fluid; mucosa normal, except a few hyperaemic patches.

Duodenum normal. Jejunum shows numerous hyperaemic patches; no follicular swelling. Ileum shows moderate swelling of Peyer's patches, and slight swelling of solitary follicles. All parts of colon show a large number of superficial erosions; colon descendens shows many hyperaemic patches.

Eight ascarides in jejuno-ileum; no other parasites.

Liver: $21 \times 11 \times 4$ cms. Capsule normal; surface dark purple, with large

yellowish patches ; tissue in some places dark, hyperaemic, but more often yellowish grey, and in some places distinctly yellow ; consistence diminished and somewhat friable.

Pancreas of normal size, intensely hyperaemic, and of diminished consistence.

Anatomical diagnoses : Hyperaemia meningum. Laryngo-tracheo-bronchitis catarrhalis. Bronchitis capillaris diffusa catarrhalis. Poly-lymphadenitis acuta. Metamorphosis adiposa myocardii et hepatis. Pancreatitis parenchymatosa acuta. Nephritis parenchymatosa acuta duplex. Hyperaemia capsulae suprarenalis sin., ventriculi et intestinorum. Haemorrhagiae lienis. Ileitis acuta. Helminthiasis intestinalis.

Histological examination :—

Liver : Intense fatty change, leucocyte groups in blood vessels.

Kidney : Moderate diffuse increase of stroma. Epithelia fairly well preserved except in a few convoluted tubules, where their protoplasm is granular and partially destroyed and the nuclei are undergoing karyolysis. The glomeruli are well preserved. Osmic acid preparations show small fat droplets in the basal portions of most of the epithelia of the convoluted tubules.

Stomach : Intense localized hyperaemia of mucosa.

Pancreas : Some parts of the sections show advanced necrobiotic changes of both the alveolar epithelia and the islets of Langerhans ; in other parts all cells are well preserved. The limit between well-preserved and necrobiotic cells is often quite sharp, cells of the two types being seen sometimes side by side in the same lobule or even in the same alveolus.

Microbiological examination :—

Nasgar tubes, fluid from lateral ventricles of the brain : Gram-negative diplococci which change glucose, galactose, and maltose, but not lactose and mannite. No growths in tubes inoculated with heart blood. No micro-organisms in smears of peripheral blood and heart blood.

Case 57. B.M., 5 years, black, seen, after death, with Dr. J. Huntly Peck, Spanish Town, March 5, 1913.

No clinical data obtainable.

Post-mortem examination, at 12 noon. Body fairly well nourished. No rigor. No jaundice. No skin affection, nor parasites.

Spinal puncture gives no fluid, even though repeated directly through the intertransversal ligaments in the lumbar and cervical regions.

Dura mater spinalis slightly hyperaemic, pia considerably hyperaemic, and dark-pigmented, especially on the sides. Cord normal.

Dura cerebralis normal, pia considerably hyperaemic, especially on the convex surface. Brain substance slightly hyperaemic.

Cervical, axillary and inguinal lymph nodules considerably enlarged and some of them hyperaemic.

Pleurae normal. Lungs slightly hyperaemic in postero-inferior parts. The smaller bronchi show slight hyperaemia of mucosa and mucous secretion ; larger bronchi, trachea and larynx normal.

Bronchial lymph nodules slightly enlarged and hyperaemic.

Thymus : $9 \times 4 \times \frac{1}{2}$ cms., of normal aspect.

Thyroid body normal.

The *pericardium* contains a few c.c. of clear fluid. Epicardium intensely hyperaemic, but shows no haemorrhages. Heart conus $5 \times 6 \times 2\frac{1}{2}$ cms. Left ventricle well contracted, forms apex. Myocardium slightly pale ; endocardium and valves normal. Pulmonary artery and aorta normal.

Diaphragm at fourth intercostal space on both sides.

Peritoneum normal. Abdominal organs normally situated.

Spleen : 9 × 6 × 2 cms. Capsule normal ; colour dark slaty ; consistence slightly diminished.

Suprarenal capsules normal.

Left *kidney :* $6\frac{1}{2} \times 4 \times 2$ cms. Capsule easily detached, surface smooth, pale, with slight distension of stellate veins ; cortex pale greyish ; pyramids normal, consistence normal. Right kidney, same characters.

Renal pelves, *ureters* and *bladder* normal ; the latter contains a considerable quantity of pale, clear urine.

Genital organs normal.

Mesenteric lymph nodules considerably enlarged and slightly hyperaemic.

Tongue coated, on its whole surface, with thick white fur.

Tonsils normal.

Oesophagus normal.

Stomach shows patches of capillary hyperaemia.

Duodenum normal. Lower down in the intestine hyperaemic patches.

Bile ducts and *gall bladder* normal, patent. Bile green.

Liver : 23 × 12 × 6 cms. Capsule normal ; colour uniform, greyish purple, tissue of normal aspect.

Pancreas hyperaemic, soft.

Hypophysis cerebri normal.

Anatomical diagnoses : Hyperaemia piae matris. Poly-lymphadenitis acuta. Bronchitis capillaris l.g. Pancreatitis parenchymatosa acuta. Hyperaemia renum l.g.

Histological examination :—

Axillary glands : Intense hyperaemia, oedema of stroma.

Spleen : Intense pigmentation.

Pancreas : Intense necrobiosis in patches, oedema of stroma.

Liver : Intense fatty change.

Kidney : Diffuse hyperaemia, more intense and with haemorrhages in some places ; beginning necrobiosis of epithelia in convoluted tubules. Oedema of stroma.

Microbiological examination :—

Cultures on nasgar (fluid from lateral ventricles of the brain) : Gram-negative diplococci, small groups, which change glucose, galactose, and maltose, but not saccharose, lactose, and mannite.

The following cases, of which only very brief notes are given, were similar to the above types of group B.

Case 1. B.D., 4 years, black, healthy from her birth until she was suddenly taken ill, on January 13, at 4 a.m. Convulsions, but no vomiting. Death at about about 7 a.m.

Post-mortem examination (with Dr. Earle, May Pen, at 6 p.m., January 13).

Spinal puncture gives about twenty drops of clear fluid. Diffuse hyperaemia of pia cerebralis, mostly pronounced on some parts of the convex surface. Slight oedema.

The *stomach* contains alimentary liquid without blood, and with no excess of mucus. Considerable hyperaemia and a few minute haemorrhages in the mucosa.

Diagnoses : Hyperaemia et oedema l.g. meningum cerebralium. Lympho-adenitis mesenterialis simplex. Hyperaemia et haemorrhagiae renum minimae.

Hyperaemia et petechiae mucosae ventriculi. Enteritis follicularis l.g. Helminthiasis intestinalis (Ascaris lumbricoides). Metamorphosis adiposa hepatis l.g.

Histological examination :—

Lymph nodules : Slight increase of stroma.

Liver : Patches of hyperaemia are seen in various parts of the sections ; the stroma around the portal vessels and the capsule are slightly increased in thickness and there is a beginning microcellular infiltration. The parenchyma is well preserved.

Kidney : Patches of hyperaemia are seen in various parts of the sections, and the stroma is slightly increased.

Suprarenal capsules : Considerable hyperaemia and marked vacuolization of cells.

Microbiological examination :—

Nasgar tubes inoculated with *spinal fluid* show growth of Gram-negative diplococci which change glucose, maltose and galactose, but not saccharose and lactose when examined in Jamaica, but in Liverpool they give different reactions which will be mentioned later on in the general discussion.

Smears from *brain*, *spleen*, and *liver* contain various bacilli and cocci.

Case 8. H.S., 3½ years, black, Post Road, by Chapelton. (Seen after death with Dr. Thomson). The child was, according to the mother, healthy until January 18, 1913, at 5 a.m., when she started vomiting and continued vomiting until death took place two hours later. No convulsions. The previous day she had eaten some unripe yam ; the mother partook of the same and had some vomiting immediately afterwards.

Post-mortem examination :—

The *stomach* contains alimentary fluid and a small amount of mucus. No haemorrhage ; no hyperaemia.

Duodenum : Slightly hyperaemic.

Diagnoses : Hyperaemia piae matris cerebralis l.g. Hypostasis pulmonum l.g. Enteritis follicularis l.g. Helminthiasis intestinalis (Ascaris lumbricoides). Lymphadenitis mesenterialis.

Microbiological examination :—

Nasgar tubes inoculated with *heart blood* remain sterile ; tubes inoculated with the sanguinolent *spinal fluid* show growth of Gram-negative diplococci which change dextrose, maltose, and to a very slight degree galactose, but not lactose, saccharose, and mannite.

Case 30. D.B., 9 months, black, Kingston Public Hospital, Dr. Thomson, January 31—February 1, 1913.

Sudden onset of vomiting and convulsions ; quite unconscious when admitted ; limbs rigid ; head retracted ; Kernig's sign marked in both legs, left more than right. No fluid obtained by lumbar puncture. Given 50 millions meningococcus-vaccine.

February 1. Rested fairly well during night. Only two attacks of convulsions no vomiting ; limbs still rigid. The child appears conscious. Temperature normal. Slight nystagmus. Slight internal strabismus, of left eye.

Post-mortem examination (performed by Dr. Thomson, who preserved the organs for me, as I was absent in the country).

Meninges congested. Lateral ventricles distended with blood-stained fluid. Upper part of spinal pia dark pigmented.

Internal organs normal.

Anatomical diagnosis : Hyperaemia meningum.

Bacteriological examination :—

Nasgar tubes inoculated with *spinal fluid* show growth of Gram-negative diplococci which change glucose, maltose and galactose, but not saccharose, lactose and mannite.

Case 33. E.B., 2 years, black, Rock, near Four Paths, with Dr. E. R. C. Earle.

Has been slightly ill for about three months. On February 3 he was unable to get up, ' he could not drink, became stiff and died at 11 a.m.' Vomiting not mentioned, nor convulsions.

Post-mortem examination : No jaundice.

Lumbar puncture gives clear, translucent fluid. Spinal cord not examined. Dura mater cerebralis normal ; pia shows moderate hyperaemia, mostly pronounced on the posterior portion of the convex surface ; no hyperaemia on the basis of the brain, but a small collection of slightly turbid fluid. The lateral ventricles are not dilated. The brain substance is of normal appearance.

Anatomical diagnoses : Hyperaemia meningum et mucosae ventriculi. Petechiae pericardii. Anaemia organorum. Helminthiasis intestinalis. Tuberculosis caseosa nodulorum lymphaticorum bronchialium.

Bacteriological examination :—

Spinal fluid, nasgar tubes : Gram-negative diplococci which change glucose, maltose and galactose, but not lactose, saccharose and mannite.

Case 43. C.B., 2 years, black, Old Hope, The Quarry, with Dr. C. R. Edwards, Half-Way Tree.

The child seems to have had slight cold for about two hours, then became very ill, vomited and died in convulsions at 5 a.m., on February 14, 1913.

Post-mortem examination, at 3 p.m., on February 14, about ten hours after death. Body fairly well nourished. No jaundice.

Lymph nodules : Axillary slightly enlarged, moderately hyperaemic ; inguinal considerably enlarged, slightly hyperaemic ; cervical moderately enlarged and hyperaemic.

Spinal puncture gives about 5 c.c. of clear, afterwards slightly turbid fluid. Spinal and cerebral durae normal, piae slightly hyperaemic, particularly on the convex surface of the brain. No exudation. Brain and cord normal. Lateral ventricles not distended.

Mesenteric lymph nodules are very much enlarged, and show punctate hyperaemia.

Spleen : 8 × 4 × 2 cms. Capsule normal, tissue normal, pink.

Anatomical diagnoses : Poly-lymphadenitis acuta l.g. Lymphadenitis mesenterialis m.g. Hyperaemia meningum. Sequelae pleuritidis sinistrae. Infarctus haemorrhagicus pulmonis dextri. Bronchitis. Enteritis l.g. Hyperaemia mucosae ventriculi

Histological examination :—

Axillary lymph nodules : Intense hyperaemia and infiltrating haemorrhages.

Mesenteric lymph nodules : Moderate hyperaemia.

Kidney : Irregularly distributed patches of hyperaemia.

Pancreas : Oedema of stroma.

Microbiological examination :—

Nasgar tubes of *spinal fluid :* Gram-negative diplococci and Gram-negative cocco-bacilli. The diplococci are isolated and are found to change glucose and maltose, but not galactose, lactose and mannite. Also the examination of the centrifugalized deposit shows Gram-negative diplococci and several other bacteria.

Case 55. A.T., 6 years, black, Barbican, St. Andrew, sent to hospital by Dr. Crosswell, Public Hospital, Kingston, March 2, 1913.

The child started vomiting on the evening of February 28, 1913, has been vomiting since then, the vomited substance being yellow. Convulsions began at 7.30 p.m. on the following day. When seen by Dr. Crosswell, all limbs were relaxed, there was no retraction of head, nor of abdominal wall; Kernig's symptom; pupils sluggish, equal; the child was semi-comatose, but could be roused, and could sit up; asked for water. He was immediately sent to hospital. On his arrival at the hospital he was moribund; the limbs were jerking, the head drawn back; temperature subnormal; skin cold and clammy; pupils dilated.

He was admitted at 8.30 p.m., and died at 9 p.m., on March 2.

Post-mortem examination, at 11 a.m., on March 3.

Lumbar puncture gives about 10 c.c. of clear fluid. Spinal meninges normal, without any hyperaemia; cord normal. Cerebral dura slightly hyperaemic; pia shows intense hyperaemia and some oedema on convex surface, slight hyperaemia on cerebellum, no hyperaemia at the basis. The lateral ventricles contain a small amount of slightly turbid fluid. The hemispheres show slight hyperaemia of the brain substance which is otherwise normal.

The *cervical and inguinal lymph nodules* are considerably enlarged and slightly hyperaemic, the *axillary nodules* considerably enlarged and intensely hyperaemic.

The *pericardium* contains a few drops of clear fluid; the epicard shows numerous petechiae and small ecchymoses on both the posterior and anterior surfaces of left ventricle, and to a less degree on the anterior surface of the right ventricle.

The *stomach* shows considerable capillary hyperaemia, corresponding to the whole length of the lesser curvature and neighbouring portions, and a few petechiae in cardiac portion; slight follicular swelling.

Duodenum slightly hyperaemic. Jejunum shows patches of capillary hyperaemia; no follicular swelling. Ileum shows hyperaemia and swelling of some of the lower Peyer's patches and considerable swelling of the solitary follicles in the last portion. Appendix 6 cms., normal. Colon normal. No parasites.

Mesenteric lymph nodules are slightly enlarged, pale, of normal consistence.

Tonsils: There is a superficial necrosis, diffuse microcellular infiltration into the connective and muscular tissues; the vessels are enormously distended.

Urine: Yellow, slightly cloudy, acid, contains no albumen, but gives a faint reaction for nucleo-albumen, it contains no sugar, no bile pigment, and no casts.

Anatomical diagnoses: Hyperaemia meningum et cerebri. Lymphadenitis multiplex. Hyperaemia et oedema pulmonum. Metamorphosis adiposa hepatis et pancreatis. Nephritis parenchymatosa acuta duplex. Ecchymoses epicardii et lienis. Hyperaemia ventriculi c. petechiis. Enteritis follicularis.

Histological examination:—

Axillary lymph nodules: There is moderate diffuse hyperaemia and some perivascular blood-infiltration.

Myocard: Sections of fragments fixed in Flemming's fluid show minute fat droplets in the muscle fibres.

Spleen: Diffuse hyperaemia.

Suprarenal capsule: Intense diffuse hyperaemia of the organ and of the surrounding connective and fatty tissues.

Kidney: Irregularly distributed patches of intense hyperaemia; the cells of the convoluted tubules are swollen and granular, with vacuoles near the basal membranes. There is a slight increase of the stroma, accompanied by a slight microcellular infiltration.

Liver: Considerable capillary hyperaemia, slight microcellular infiltration of stroma and penetrating amongst the liver cells, which show advanced fatty change mostly marked in the portal and intermedial zones.

Stomach: The sections show the transition from the oesophagus to the stomach. The oesophageal epithelium is fairly well preserved. The gastric epithelium on the contrary is to a very large extent destroyed and in some place completely destroyed. There is an intense hyperaemia, and in the gastric mucosa also minute haemorrhages. The gastric lymphoid follicles are enlarged and contain numerous polymorphonuclear leucocytes and there is also diffuse microcellular infiltration of both mucosa and submucosa of the stomach, whilst the same phenomena are but slightly pronounced in the oesophagus. There is some oedema of the stroma. Numerous 'mast-zellen' in all coats, especially of the oesophagus. Bacteria are exceedingly numerous in the superficial portions of the stomach, and in some places groups of bacteria are observed in the deeper layers in blood vessels.

Centrifugalization-deposit of the *spinal fluid* shows numerous endothelial cells, few lymphocytes and a few polymorphonuclear leucocytes.

Microbiological examination:—

Spinal fluid, cultures on nasgar: Gram-negative diplococci which change glucose, maltose and galactose, but not saccharose, lactose and mannite. No micro-organisms are seen in smears from spinal fluid and blood from lateral ventricles.

Group C contains cases resembling those in the two preceding groups in their clinical and anatomical characteristics, but no bacteriological examination of the cerebro-spinal fluid has taken place. In fact, the cases in the three groups, A, B and C, are often so much alike that the only constant difference is that in B meningococci have been demonstrated, in C they have not been examined for, and in A they have not been found, though carefully examined for. A satisfactory classification of the cases in this group cannot be attempted until the Groups A and B have been discussed. Group C contains 11 cases, namely, Cases 2, 5, 10, 11, 13, 18, 24, 25, 27, 32 and 48.

The cases in this group are given in abstracts only.

Case 2. R.B., 4 years, coloured (seen with Dr. Earle, May Pen), January 13. Lives in the house next door to the one in which Case 1 died, and was reported ill whilst the autopsy on this case was being performed. The child started vomiting at 7 p.m., and was seen at 9 p.m., when it was in a state of slight collapse, with soft and somewhat irregular pulse, about 90. It was stimulated with ether, and improved rapidly. Recovery.

This case was, according to Dr. Earle, one of so-called 'vomiting sickness.'

Case 5. A.A.J., 23 months, black, male, not seen by physician before death. Melrose.

Taken ill suddenly on January 15, at 5 a.m., with pain in stomach, and efforts of vomiting, but no actual vomiting. No convulsions. Death took place at 7 a.m.

Post-mortem examination, with Dr. Cooke, Mandeville.

The *kidney* measures 6 × 3½ × 1½ cms. Capsule easily detached. Surface smooth, with minute haemorrhages and considerable hyperaemia. Renal substance reddish grey, almost uniform throughout cortex and pyramids. A few minute haemorrhages and yellowish patches in the substance.

Liver: 16 × 11 × 4 cms. Capsule smooth and transparent. Substance uniformly greyish red, of normal consistence. No yellow, or soft patches.

Pancreas of normal size, colour, and consistence.

Oesophagus, *stomach*, and *duodenum* without pathological changes. No hyperaemia except very slightly in the duodenum. The stomach contains a small quantity of liquid food; no blood.

Jejunum of normal characters, except for a slight enlargement of the solitary follicles in its upper part. A few of the last Peyer's patches in the *ileum* are slightly enlarged.

Appendix, *coecum*, and *colon* normal. No intestinal parasites.

Mesenteric lymph nodules not enlarged.

Diagnoses: Hyperaemia meningum l.g. Tonsillitis chronica. Nephritis acuta l.g. Enteritis follicularis l.g. Splenitis acuta l.g.

Case 10. S.S., 4 years, brown, 4 Chancery Lane, seen with Drs. Dryden and Thomson in the Public Hospital, Kingston. January 21, 1913.

Admitted with her playmate, Case 9 (group A). Symptoms and course similar, but weakness less pronounced.

Microscopical examination of the *blood* shows no parasites.

Case 11. A.W., 2 months, black, 82 Ruin Lane, seen in Public Hospital, Kingston, with Dr. Thomson. Admitted January 21, 1913.

Admitted to hospital on account of continuous vomiting and collapse; has been ailing for one day. Breast-fed. No convulsions, and no other meningeal symptoms. Temperature 100° F. in rectum. Gums swollen and tender.

The following days no vomiting, and no other symptoms. Temperature normal.

Microscopical examination of the *blood* shows no parasites.

Case 13. E.S., 16 years, black, Four Roads (seen with Dr. Croswell). The patient was well until the morning of January 23, when she was taken ill, feeling unwell and vomiting twice during the day. She was not feverish. The following morning she felt very weak, and was taken to Dr. C's surgery where she vomited once. This vomit was watery, frothy, but those of the previous day were described as 'bilious.' The temperature was normal, the pulse somewhat weak. Dr. C. called me to see the patient, and I saw her with Dr. C. at noon. She looked normal, slightly drowsy. Pulse 92, slightly soft and irregular. No jaundice. Heart, liver and spleen-dullness normal. No abdominal pain, tenderness or contraction. No stiffness of neck or other muscles. No Kernig's symptom. No deviation of eyes. Numerous rhagadae on the lips, and a herpes-like eruption on the chin.

No parasites were found in blood smears.

(This case was considered by Dr. Croswell to correspond fairly well to the picture of 'vomiting sickness').

In the following case there were several attacks of 'meningismus,' during the course of a suppurative infection. When the child died, the possibility of a purulent meningitis was therefore considered, but both the post-mortem findings and the final part of the illness were in accordance with the type of vomiting sickness.

Case 18. A.B., 4 months, black, Bloxburgh, St. Andrew. Seen at Kingston Public Hospital, with Dr. C. A. A. Thomson. January 4—29, 1913.

January 4, admitted, with abscess of axilla, observed three weeks previously; is said to have had convulsions recently. No vomiting, nor diarrhoea. Abscess incised. Temperature normal.

January 14. Has had several attacks of convulsions. No rigidity; no Kernig's symptom. Bowels free. Incision wound healing.

January 17. No convulsions for the past 48 hours. Wound healed, but there is some hard infiltration of the surrounding parts. Temperature normal.

January 29. Return of convulsions yesterday, one lasting several minutes followed by exhaustion and death.

Clinical diagnosis: Posterior basal meningitis.

Post-mortem examination: (29 January, 1913).

Anatomical diagnoses: Atrophia universalis. Anaemia organorum. Hyperaemia piae matris cerebralis. Pediculosis capitis.

Case 24. M.E., Craigmill, black, adult, seen with Dr. George.

The patient had complained of cardialgia and nausea for several days. On January 29, she started vomiting at about 2 p.m.; she had no fever. She went on vomiting for two days, bringing up considerable, according to description enormous, quantities of watery, yellow or greenish fluid. Temperature normal. Pulse 92, regular, weak. No meningeal symptoms.

February 1. Patient admitted to hospital. No vomiting. Pulse and general condition improved, after tinct. strophanti.

Case 25. R.M., 10 years, black, Craigmill. Seen on January 31, 1913, with Dr. George.

Patient has vomited four times since morning; was seen by Dr. George at 4 p.m. There is some epigastric pain and tenderness, when he is seen by Dr. G. and myself at 11.30 p.m. Pulse fairly good, but not strong. No vomiting since this afternoon. No meningeal symptoms.

February 1. Improved.

Recovered.

Case 32. E.B., 6 years, black, Rock near Four Paths, with Dr. Earle. 'Quite well on the morning of February 3. In the afternoon she vomited greenish substance and went to bed. About midnight she again started vomiting and continued till daybreak of the 4th. The jaw was locked, she could not swallow, became stiff and ground her teeth.'

The patient died at 10 a.m., on February 4, 1913.

Post-mortem examination :—

Liver: 18 × 10 × 4 cms. Capsule normal; surface and substance uniformly reddish grey.

The *stomach* contains clear alimentary fluid; the mucosa shows, on both sides of the lesser curvature capillary hyperaemia and numerous petechiae.

The *jejunum* and *ileum* contain numerous ascarides; no other intestinal parasites. The Peyer's patches in the lower part of ileum are slightly enlarged and hyperaemic.

The *pancreas* is normal.

Anatomical diagnoses: Hyperaemia piae matris cerebralis. Hyperaemia et petechiae ventriculi. Enteritis l.g. Nephritis parenchymatosa acuta l.g. Helminthiasis intestinalis.

Case 48. L.W., 12 years, black, Windward Road, Doncaster Pen. Seen at Public Hospital, Kingston, with Dr. Thomson.

Admitted, on February 18, 1913, with history of sudden onset of vomiting the previous night. An aunt (case 47) and her infant were similarly attacked, the infant dying at home.*

Temperature on admission normal, and remained so. Patient drowsy. No vomiting. No muscular rigidity; no Kernig's symptom.

February 19, rested well last night. No malarial parasites in blood.

Patient recovered, and left Hospital on February 21; he was then apparently quite well, but the pulse was very frequent, 100—120, weak and slightly irregular.

Case 51. E.E.E., 20 years, black, seen with Dr. Croswell, on February 23; post-mortem examination with Dr. Edwards on February 24.

The patient was taken ill suddenly, on February 22, at 7 a.m. with convulsions, was seen by Dr. C. in the afternoon, was then very violent; after chloral and bromide she seemed to improve, though she had repeated convulsions. The following day, just after noon, she became unconscious, and comatose, and was in a dying condition, when seen on February 23, at 4 p.m. by Dr. C. and myself. Temperature was then 105° F., pulse could not be felt; she reacted a little, when given injections of ether and digitalin, so that the pulse could just be felt, filiform, at the radials, but not counted; the heart beats were weak, about 150 to 180. She died in coma at about 5 p.m.

When seen just before death, the pupils were of normal size; there was no retraction of the head, but slight rigidity of the neck-muscles. No Kernig's sign. Abdomen natural, no enlargement of liver, or spleen. Duration of illness: 34 hours.

Post-mortem examination, at 12 noon, on February 24, about 19 hours after death.

Body well nourished, healthy looking. Excoriations on the lips. Rigor present. No jaundice.

Cervical, inguinal and particularly the *axillary lymph nodules* considerably enlarged and intensely hyperaemic.

No spinal fluid obtained by lumbar puncture. Cerebral dura and pia without hyperaemia and exudation; lateral ventricles not distended; brain substance normal. Spinal dura, pia and cord perfectly normal in dorsal portion, the only part examined.

Hypophysis cerebri: Normal.

Pleurae: Left pleura shows numerous small ecchymoses, especially on the inferior lobe.

The *pericardium* contains a few c.c. of clear fluid; the epicard shows diffuse hyperaemia and numerous small ecchymoses on the posterior surface of the left

* A post-mortem examination was made on the infant by Dr. Gifford who informed me that he had found no hyperaemia of meninges and no pronounced lesions of any organ.

ventricle and auricle. Heart conus 9 × 10 × 5 cms.; apex is formed by left ventricle which is somewhat flabby. Endocard and valves normal. Myocard pale, with yellowish patches, of rather soft consistence.

Mesenteric lymph nodules slightly enlarged and hyperaemic.

Spleen: 10 × 8 × 2 cms. Capsule slightly thickened, whitish; colour of tissue normal; follicles not prominent, but a considerable number of small (from miliar to lenticular) yellow nodules are seen in the pulpa. Consistence normal.

Suprarenal capsules normal.

Left *kidney:* 12 × 6 × 3 cms. Capsule slightly adherent; surface smooth, except along some entering vessels, where capsule is adherent; surface pale yellowish, with patches of capillary hyperaemia; cortex pale, yellowish, with prominent glomeruli and some hyperaemic stripes; columnae Bertini still more yellow and rather soft; their tissue is bulging forward on the cut surface; the pyramids show slight hyperaemia of their bases.

Right *kidney:* 12 × 7 × 3 cms. Shows same characters as left.

Renal pelves, ureters and bladder hyperaemic. The calyces, pelvis and ureter on the right side contain a yellowish, purulent fluid. The bladder contains about 100 c.c. of pale, slightly turbid urine.

Both *ovaries* are hyperaemic; the right contains a fresh corpus luteum and the left also one, apparently of recent date. Both contain, besides, small serous cysts. The Fallopian tubes are not distended; only the left is patent; its mucosa is apparently normal, but the ovarian extremity contains purulent fluid. Uterus hyperaemic; its cavity measures, from orificium internum to fundus 4 cms.; collum 3 cms. Endometrium corporis hyperaemic and covered with slight purulent secretion. The cervix contains muco-pus; its mucosa is swollen, with minute haemorrhages. The portio vaginalis is discoloured, dark, with erosions.

Liver: 30 × 16 × 8 cms. Capsule normal; surface spotted red and yellowish grey, with subcapsular hyperaemia and minute haemorrhages; the tissue is uniformly yellowish grey; structure enhanced by slight hyperaemia of portal veins; consistence soft and extremely friable.

Pancreas of normal size, considerably hyperaemic and very soft.

Tonsils show small purulent particles on the surface, not enlarged.

Oesophagus: Slightly hyperaemic, contains greenish black fluid.

Stomach considerably distended, contains greenish black fluid with small black particles; no smell of phosphorus. The mucosa shows considerable hyperaemia of the cardiac portion, on both anterior and posterior walls, and a large number of petechiae; on the remaining parts of the mucosa a few small erosions are seen.

Duodenum shows hyperaemia and some petechiae.

Appendix 17 cms., normal.

Bile ducts and gall bladder normal; the former are patent, and the latter contains a moderate quantity of greenish bile.

Urine: Turbid; acid, contains about 1 per mille albumen and a few granular casts, no indican, no bile pigments and no sugar.

Anatomical diagnoses: Poly-lymphadenitis acuta. Haemorrhagiae pleurae sinistrae, pericardii, et capsulae hepatis. Hypostasis pulmonum. Laryngo-tracheo-bronchitis catarrhalis. Struma simplex l.g. Metamorphosis adiposa myocardii et hepatis m.g. Pancreatitis parenchymatosa acuta. Nephritis parenchymatosa acuta duplex m.g. (in chronica). Pyelo-ureteritis dextra. Endometritis. Salpingitis. Hyperaemia ovariorum. Noduli lienis. Gastritis ecchymotica cum erosionibus. Duodenitis ecchymotica. Hyperaemia jejuni et coli. Pharyngitis acuta.

Histological examination :—

Axillary lymph nodule : Intense hyperaemia and haemorrhages, both arteries, veins and capillaries being enormously distended ; there is diffuse erythrocyte infiltration of the tissues especially in the central portion of the nodule.

Liver : Intense fatty change and slight necrobiotic changes equally distributed throughout the lobuli ; several patches of capillary hyperaemia.

Pancreas : In the sublimate-alcohol specimens, only slight localized necrobiotic changes are observed, but in the Flemming specimens there is marked diffuse fatty metamorphosis present, all or nearly all cells containing minute fat droplets.

Kidney : The glomeruli are distended and some of them hyperaemic. Most of the epithelial cells in the convoluted tubules contain large vacuoles (fat, Flemming). A few tubules show marked necrobiotic changes of the cells. The vacuoles, when not filling the cell entirely, always occupy the basal portion of the cell ; as a rule the cell contains only one or a few fat drops. In some portions of the kidney the necrobiotic changes are more marked and the fatty metamorphosis less marked. In these portions there is some capillary hyperaemia. There is a slight increase of the connective tissue throughout the sections ; there is no microcellular infiltration.

Microbiological examination :—

No growth in nasgar and haemoglobin agar tubes inoculated with heart blood, except some colonies of large bacteria, probably accidental infection. No parasites in smears of peripheral blood and heart blood. No gonococci in smears from the right renal pelvis and endometrium.

In group D we include the cases in which the usual characteristics of meningococcus-meningitis were found, namely definite inflammatory changes in the meninges and meningococci in the cerebrospinal fluid. The following five cases fulfil these requirements : 4, 19, 29, 31 and 39. All these cases are given in abstract only.

Case 4. V.C., 2 years, female, black, of Craigmill, Portland, seen, after death, with Dr. H. J. George, of Buff Bay.

The child was taken ill at 5 p.m. on January 13, having been in good health previously. She had never before suffered from convulsions, fever, or vomiting. She started vomiting and continued vomiting, apparently without any effort, until her death which took place at about 5.30 p.m., i.e., about half an hour from the onset. The child did not appear feverish, there were no convulsions, and no stiffness of neck or other muscles was noticed.

Post-mortem examination was made at 6 p.m., on January 14, i.e., 25 hours p.m. Well nourished child. No jaundice.

Spinal puncture gives about twenty drops of clear, yellowish fluid.

Meninges : Intense congestion of both cerebral and spinal dura and pia, but mostly pronounced in the pia on the convex surface of the brain, and on the upper surface of cerebellum. Slight oedema of pia. Beginning sero-fibrinous exudation in median and Sylvian fissures.

Brain and spinal cord show, in some parts, irregularly distributed slight hyperaemia, but no other macroscopical lesions.

Mouth, *pharynx*, *tongue*, *tonsils*, *larynx*, *trachea* and *bronchi*, *thyroid body* and *thymus* are macroscopically normal. No liquid and no adhesions in *pleurae*.

Oesophagus, *stomach* and *duodenum* without pathological changes.

Liver : 17 × 10 × 4 cms. Capsule smooth, transparent. Colour slightly irregular, greyish with red patches. No yellow colouring except a few small patches in the left lobule. Substance slightly pale, otherwise normal.

Pancreas : Of normal size, colour and consistence.

Anatomical Diagnoses : Hyperaemia diffusa meningum. Leptomeningitis cerebralis fibrinosa incipiens. Hypostasis pulmonum l.g. Infarcti haemorrhagici pulmonis sinistri. Anaemia myocardii. Anthracosis lymphonodulorum bronchialium (et tuberculosis ?) Cystis haemorrhagicus ovarii dextri. Lympho-adenitis mesenterialis simplex. Enteritis follicularis l.g. Helminthiasis intestinalis (Ascaris lumbricoides).

Microbiological examination :—

Nasgar tubes, *spinal fluid :* Gram-negative diplococci which change glucose and maltose, but not galactose, lactose and saccharose.

Case 19. A.H., 4 years, black, 12 Beeston Street, Kingston. At Kingston Public Hospital (Dr. Thomson), January 18, 1913

Apparently well till January 17, at 11 p.m., when he started vomiting ; he had convulsions, was stiff at times ; the head was drawn back.

He was admitted to hospital early the following morning, and died at 8.30 a.m., on January 18.

Kernig's sign was present on both sides.

Lumbar puncture gave spinal fluid under considerable pressure ; the fluid was clear.

The urine was clear, contained no albumin, and no casts.

Post-mortem diagnoses (Dr. Scott) : Hyperaemia piae matris. Meningitis sero-fibrinosa. Sequelae pleuritidis sinistrae. Lymphadenitis mesenterialis. Metamorphosis adiposa hepatis, l.g.

Microbiological examination :—

Nasgar tubes, *spinal fluid :* Gram-negative diplococci which change glucose, maltose and galactose, but not lactose, saccharose, and mannite.

Case 29. B.W., 12 years, male, black, admitted to Kingston Hospital, January 25, 1913.

History of ' fever ' ; delirium on admission ; temperature 100° F. ; no retraction ; no rigidity ; no Kernig ; spleen not felt ; passes urine in bed ; illness said to have lasted a week or longer. Diagnosis of malaria ; blood shows crescents. January 26 :—temperature 101° F., still unconscious, had two attacks of convulsions during night. Died January 28, 9.20 a.m.

Post-mortem examination was made at 2 p.m. (by Dr. Scott), on January 28, i.e., 5 hours p.m.

Thoracic organs : Nothing abnormal.

Spleen is dark, greyish-black, not much enlarged. 5 × 3 × 1 cms.

Liver is dark, greyish-black.

Congestion of *Pia* and surface of Brain is very marked ; thickish lymph over cortex, with purulent patches between hemispheres ; at base, greenish-yellow pus and abundant turbid fluid. Ventricles distended, about 5—6 ozs. of slightly turbid fluid in each. Spinal fluid turbid and abundant.

Anatomical Diagnoses : Leptomeningitis, fibrino-purulenta. Splenitis acuta Hepatitis parenchymatosa.

Microbiological examination :—

Spinal fluid : Smears show numerous intracellular diplococci ; cultures on nasgar show colonies of Gram-negative diplococci which change glucose, maltose and galactose, but not lactose, saccharose and mannite.

Case 31. H.M., 19 months, black. At Kingston Public Hospital, February 3—5,1913.

Admitted unconscious, on February 3, at 3.45 p.m. History of vomiting and convulsions of sudden onset.

Temperature 98. Slight strabismus. Limbs rigid. Lumbar puncture: no fluid. February 4, Temperature 102. Has vomited once. Head retracted. Kernig's sign doubtful. Knee-jerks not obtained.

February 5, very weak. Cheyne-Stokes respiration. Death.

Post-mortem examination (performed by Dr. Scott, who preserved the organs for me, as I was out in the country).

No congestion of meninges and brain, but lateral ventricles distended with turbid fluid. Inflammatory lymph at basis of brain. Spinal canal shut off by a layer of lymph like a diaphragm (neg. res. of lumbar punct. !) Internal organs normal.

Anatomical Diagnosis : Meningitis.

Microbiological examination :—

Nasgar tubes, *spinal fluid :* Gram-negative diplococci which change glucose, maltose and galactose, but not saccharose, lactose and mannite.

Case 39. U.M., 8 years, black, Osborne Store, Four Paths, Clarendon, with Dr. E. R. C. Earle, May Pen.

The child was healthy until Sunday, February 9, 1913, when he was taken ill with vomiting, at about 9 a.m. He improved after mint-tea and whisky, but on the following night vomiting started again, at about 3.30 a.m. on February 10. He vomited repeatedly during the day. He was seen by Dr. Earle at about 5 p.m., but was then already in a dying condition. He died at 6 p.m.

Post-mortem examination :—

Cervical, axillary and inguinal lymph nodules slightly swollen.

Lumbar puncture gives clear fluid. Dura mater normal. Pia mater slightly hyperaemic on the convex surface of the brain, more pronouncedly so on the basis and especially on the cerebellum; it appears normal on the upper part of the spinal cord, but there is a slight hyperaemia of its lumbar portion. There is a small amount of slightly clouded, serous exudation at the basis of the brain, of which liquid smears are taken. Lateral ventricles of normal aspect, as well as the substance of brain and cord.

The *stomach* is somewhat dilated and contains a considerable quantity of fluid of the character of intestinal contents. The gastric mucosa is normal in its larger part, but shows in its central portion, on the anterior surface and close to the lesser curvature, a large hyperaemic patch with several petechiae and a few petechiae scattered about on the neighbouring parts. Near the pylorus, on the posterior wall, considerable follicular swelling is observed. The duodenum shows hyperaemia, ecchymoses and follicular swelling. The jejunum is hyperaemic, especially in its upper portion; no haemorrhages. The intestinal tract is otherwise normal; it contains no parasites.

Anatomical diagnoses : Leptomeningitis incipiens. Lymphadenitis simplex l.g. Hypostasis et oedema l.g. pulmonum. Bronchitis. Metamorphosis adiposa myocardii et hepatis. Gastritis ecchymotica et follicularis. Enteritis l.g. Splenitis et perisplenitis chronica. Pancreatitis parenchymatosa acuta. Nephritis parenchymatosa acuta l.g.

Urine : Faint albumin-reaction and hyaline casts.

Histological examination :—

Spleen : Oedema of stroma.

Myocard: Fragmentation.

Pancreas: Extensive necrobiotic changes, most nuclei remaining unstained, and the cell limits seldom being recognisable.

Stomach: The mucosa shows considerable hyperaemia and some superficial haemorrhages, and diffuse microcellular infiltration.

Smears of spinal fluid after centrifugalization show a number of lymphocytes and a few polymorphonuclear leucocytes.

Microbiological examination:—

Nasgar tubes, *spinal fluid:* Gram-negative diplococci which change glucose, maltose and galactose, but not lactose, saccharose and mannite. Same result on haemoglobin agar. *Heart blood:* Growth of Gram-negative bacteria which ferment all sugars tested.

In smears from *liver*, chains and groups of diplococci are observed, some of them being situated in mononuclear leucocytes. In smears of spinal fluid and heart blood no cocci and no protozoal organisms are observed.

Personally, I saw no cases of cerebro-spinal meningitis of an ordinary type, but Scott described (1913), as already mentioned, several such cases in connection with his investigations on 'vomiting sickness.' As a further proof that *Diplococcus meningitidis* occurred in Jamaica at the same time that 'vomiting sickness' was prevalent, I give abstracts of two observations, for which I am indebted to Colonel Wilson, R.A.M.C.

Case I. This was a case in a Sergeant-Major who was taken ill on the 2nd January, 1913, and died on the 6th. Clinical symptoms of meningitis were present and there was a rise of temperature during the first days of the disease, with an intermission on the 3rd and 4th day and a terminal rise up to 107·2 just before death. The post-mortem examination showed an incipient meningitis, the cerebro-spinal fluid being turbid 'almost purulent.' The spinal fluid contained Gram-negative diplococci which did not grow on human Pleuritis serum.

Case II. This case occurred in the Chaplain of the Forces who was taken ill on the 15th January, 1913. He presented marked clinical symptoms of meningitis, and the disease lasted for about ten days. The patient recovered. Meningococci were not demonstrated in the spinal fluid but developed in cultures from the naso-pharynx.

Group E is composed of three cases, in which anatomically a commencing meningitis was present, but no meningococci were found. In Case 17 an adult woman died after an illness lasting for about 60 hours, during which she had presented symptoms fairly typical of 'vomiting sickness'; her five years' old child was taken ill the same day as the mother, and died within six hours. The child did not come under my observation, but I am indebted to Dr. Edwards for the notes which are given below. The other patient in this group, Case 35, was a girl of 11 years, living in the same house as Case 36,

a boy of 11. Both children were taken ill at the same time, with the same symptoms, and the duration of the illness was nearly the same in both cases. All these circumstances evidently point to the same disease having attacked the two children; in spite of all, the two cases must be classified under different headings, as the results of the post-mortem examinations, which were performed at the same time, differed considerably. In Case 35 there was a marked, though commencing inflammation of the meninges, but apparently no pathological changes in the stomach or intestines. In Case 36, on the other hand, there was a well marked haemorrhagic gastritis, whilst the meninges showed only slight diffuse hyperaemia, a common phenomenon in acute infectious diseases. Case 36 is therefore discussed as belonging to the following group of cases 'suspicious of yellow fever,' whilst Case 35 is considered, as it anatomically must be considered, as one of meningitis. In the latter case staphylococci were cultivated from the spinal fluid, but it appears extremely unlikely that this occasional occurrence of a very common organism should be of any etiological importance. All circumstances considered, it is probably correct to regard the two cases, 17 and 35, as meningitis of unknown origin, but belonging essentially to the same disease as is represented by the cases.

Thus, it may be that the separation of the two cases from those in Group A occurring in the same places and under identical circumstances is only provisional. Case 62 also somewhat resembles the type in Group A.

Case 17. M.H., 27 years, St. Andrews. Admitted January 29, 3.10 p.m. Public Hospital, Kingston, having vomited for the past 36 hours, complaining of headache and pain in the limbs.

Whilst in hospital, no vomiting occurred, but patient had some mucopurulent expectoration. Temperature 99°. Pulse about 80—90, regular and full to begin with, apparently with somewhat increased tension, but on the morning of January 30 it was weak and irregular. She was conscious during the first day, but the last morning she was in a state of quiet delirium and gradually passed into coma. There were no shrieks, but some grinding of the teeth. No muscular rigidity, and no Kernig's symptom. Knee-jerk slightly increased on the left side, but could not be produced on the right. No convulsions.

Spinal puncture made on January 30, about noon. Clear watery fluid comes out, rapidly dripping; about two c.c. taken.

Patient died at 2.15 p.m., January 30.

Post-mortem examination: (With Dr. Scott) at 3.30 p.m., on January 30.

Intense hyperaemia of *cerebral pia*, less pronounced of spinal pia. Some oedema and beginning fibrinous exudate over the cerebral hemisphere, not on the basis, nor on the spinal cord.

The *stomach* shows slight oedema of the mucosa, and in some parts slight capillary hyperaemia ; a pin head-sized superficial ulcer on the posterior surface, near the lesser curvature.

Anatomical diagnoses : Leptomeningitis cerebralis incipiens. Metamorphosis parenchymatosa et adiposa myocardii. Ulcus ventriculi parvum. Gastritis acuta l.g. Nephritis acuta parenchymatosa. Perisplenitis chronica fibrosa. Metamorphosis adiposa et parenchymatosa hepatis. Pancreatitis parenchymatosa acuta. Helminthiasis intestinalis.

Microbiological examination :—

Nasgar tubes, *spinal fluid :* remain sterile.

This patient was the mother of a child which was taken ill and died on January 28th, i.e., the same day that the mother's illness began. This case was observed by Dr. Edwards, to whom I am indebted for the following particulars, which show that the case, if classified, would have been included in Group A.

L.F., 4 years, 10 months, was taken ill at 4 p.m. Vomited several times convulsions set in and coma and death supervened at 10 p.m. Dr. Edwards performed the post-mortem examination and found :

Conjunctivae slightly jaundiced. Pleurae, lungs, heart, pericardium, peritoneum, liver and spleen normal. Kidneys pale, the stroma show slight conjugation of the cardiac portion, no blood in the lateral ventricles of the brain. Meninges not congested.

Nasgar tubes were inoculated at the post-mortem examination with the *spinal fluid*. They remained sterile.

Case 35. E.M., 11 years, black, Tollgate, with Dr. Earle. Living in same house as No. 36. May Pen.

The child was healthy until Tuesday, February 4, 1913, at about 3 p.m., when she started vomiting some black substance, first declared so by grandmother, who observed the child, but on further questioning she declared that the vomiting had been colourless ; she continued vomiting until the following afternoon, when the vomits ceased, and soon after convulsions, retraction of the head, and contraction of the jaw were observed. She died at 4 p.m., on the 5th, without medical attendance.

Post-mortem examination :—

Dura mater cerebralis normal. Pia mater shows slight diffuse hyperaemia and oedema, and beginning fibrino-purulent exudation around some of the vessels on the convex surface. Brain substance of normal appearance.

Anatomical diagnoses : Leptomeningitis cerebralis incipiens. Lymphoadenitis mesenterialis simplex. Splenitis acuta. Nephritis acuta. Pancreatitis parenchymatosa acuta. Helminthiasis intestinalis. Cadaverositas universalis.

Microbiological examination :—

Spinal fluid : Nasgar tubes, no growth of diplococci, but of Gram-positive staphylococci.

Case 62. R.A.L., 9 years, black, seen, after death, with Dr. C. R. Edwards, at Kingston Public Hospital, March 9, 1913.

Post-mortem examination :—

Lumbar puncture gives a few c.c. of clear fluid, with admixture of blood. Cerebral sinuses distended with blood clots ; pia considerably hyperaemic, especially on convex surface and with slight sero-fibrinous exudation at the basis. Lateral ventricles not distended, contain a very small quantity of serous fluid. Spinal dura normal ; pia very slightly hyperaemic in lumbar portion and slightly pigmented. Brain and cord normal.

Urine : Contains traces of albumin and nucleo-albumin.

Anatomical diagnoses : Leptomeningitis cerebralis incipiens. Splenitis acuta. Enteritis follicularis. Nephritis parenchymatosa acuta l.g. Petechiae epicardii. Infarcti haemorrhagici pulmonis dextri. Sequelae pleuritidis duplicis. Adenitis bronchialis caseosa. Endarteriitis aortae incipiens. Helminthiasis intestinalis. Pediculosis capitis.

Histological examination :

Cerebral basis : The pia mater shows microcellular infiltration, no organisms are observed.

Spleen : Considerable hyperaemia.

Liver : Moderate fatty change, oedema of stroma.

Pancreas : Both the alveolar epithelia and the islets of Langerhans of normal appearances. The latter in particular are very well preserved.

Kidney : The epithelia of the convoluted tubules show granulation metamorphosis, and slight necrobiosis, also slight vacuolization in the basal portions of many of the cells. In osmic acid preparations minute fat droplets are seen, chiefly in the basal portions of the epithelia of the convoluted tubules, but also in other parts of these cells and in other cells, the epithelia of Bowman's capsules and the glomerulus-cells also being attacked.

Duodenum : Considerable microcellular infiltration of the mucosa. The lymphoid follicles are considerably enlarged, numerous mast cells are present.

Lung infarct : alveoli filled with blood of apparently normal composition, slight epithelial desquamation and microcellular infiltration of stroma in neighbouring parts.

Microbiological examination :—

Nasgar tubes inoculated with *spinal fluid* and *heart blood* remain sterile.

Group F is very important. It contains cases in which one or several symptoms gave rise to a suspicion of yellow fever. The suspicious symptom which has been chiefly considered is black vomit. Petechiae in the gastric mucosa may easily occur in other conditions, and fatty liver is common in acute infections in children. Therefore, these two phenomena have not been considered sufficient to justify the inclusion of cases in this group, not even if supported by the presence of acute nephritis. All these lesions are so common in 'vomiting sickness,' that they must really be considered in the general discussion. On the other hand, dealing with extremely acute cases, the possibility must be admitted that jaundice may not have had time to develop. As it, moreover, is known that jaundice is not invariably present even in fatal cases of yellow fever, the

presence of this symptom was not considered necessary in order to include a case in this group. It is true, of course, that also black vomit is no absolutely constant symptom, but it would not be likely that it should be absent in rapidly developing and fatal cases. The observation of black stomach contents at the post-mortem examination is regarded as equivalent to the occurrence of black vomit. In this way, and considering the general characters, the following five cases were regarded as suspicious: Nos. 23, 36, 38, 47 and 54. Notes of all these cases are given below for reference. In Case 23 there is little doubt that yellow fever is the correct diagnosis. No complete clinical history could be obtained, as the patient, a Chinaman, was unable to answer questions when I saw him, and his Chinese friends explained themselves somewhat poorly in English. Thus we could not find out whether the patient had suffered from febrile symptoms, though it was evident that he had complained of severe headache. It was also ascertained beyond doubt that black vomit had been present, and the same could be concluded from the aspect of the tongue. Examination of the blood showed absence of malarial parasites, but a few intracorpuscular bodies were found of the type of *Paraplasma flavigenum*. The post-mortem findings were typical of yellow fever in the essential details; the small whitish patches which were found in the cerebral pia mater were possibly remnants of some old meningeal affection, but there was no fresh inflammation. The appearances of stomach, liver and kidney were those met with in yellow fever. The liver showed, microscopically, marked fatty and necrobiotic changes, in some places of the Rocha-Lima type.* No meningococci developed in the cultures, but diplococci were found in smears from various organs, an observation which will be referred to later on.

The anatomical findings in Case 36 are very suspicious of yellow fever, in spite of the absence of jaundice. Considering, however, that gastric and pericardial petechiae were frequently observed also in cases of 'vomiting sickness' which were not suggestive of yellow fever, I should not feel confident in making

* Rocha-Lima (1912) described a type of necrobiosis, combined with fatty change, in the liver, which he considers characteristic of yellow fever; the essential characteristic is that the necrobiotic changes are mostly pronounced in the intermediate portions of the lobules. This type is, according to my experience, common but not constant in yellow fever.

a diagnosis of yellow fever in such a case in the absence of a typical clinical history and of an examination of blood taken during life.

Case 38 is similar to Case 36, with the additional symptom of jaundice, thus strengthening the suspicion of yellow fever, but even thus the evidence cannot be regarded as conclusive.

The Cases 47 and 54 are also essentially similar. The symptoms are not suggestive of yellow fever, but rather of a meningeal affection, though not markedly so. In Case 54, however, black vomit was observed; in this case the peripheral blood was examined, and no *Paraplasma flavigenum* was found.

All these cases illustrate my previous statements about the difficulties which beset the post-mortem diagnosis of yellow fever. In all four cases the pathological lesions point to this diagnosis, but the clinical and epidemiological characters make one hesitate in pronouncing it, in the absence of conclusive parasitological evidence.

Case 23. C.C., male, about 20 years, Chinese, Charlestown. Seen, on January 31, 1913, with Dr. H. J. George.

The patient had lived in Jamaica from when he was a boy, and had been in good health up till present illness.

He felt ill on January 27, started vomiting at about 10 a.m., to begin with yellowish, later on greenish, and on January 30 and 31, red and black. He was seen by Dr. George on January 31, in the morning; the temperature was then normal. An injection of meningococcus-vaccine was given. Near midnight, on the same date, I saw the patient, together with Dr. George. He was then in a semi-comatose condition, not answering questions. Temperature was normal, and it was not quite clear whether he had been febrile, but it was stated that he had suffered from headache, besides the vomiting. Pulse about 90, weak, and slightly irregular. Respiration stertorous. No enlargement of spleen or liver. No abdominal tenderness. Perhaps slight jaundice of conjunctivae. The tongue has red borders, but is otherwise covered with a blackish fur.

On February 1, at 8.30 a.m., the patient arrived at the hospital in Buff Bay, in an extremely weak condition. The temperature was not taken, but the skin felt very cold to the touch. Pulse was filiform. There was no vomiting, but enema provoked a greenish-black evacuation. The patient did not react on stimulating treatment, and died at 11.30 a.m.

Post-mortem examination, at 2 p.m., 2½ hours after death.

Well nourished, fat body. Rigor present. Livores are very extensive on back and sides, and are also present on the front of the femora. Slight jaundice.

Spinal puncture gives blood-stained fluid. Spinal cord not examined.

Brain. Dura intensely hyperaemic, pia likewise hyperaemic, but less pronouncedly. No liquid exudate, but on the convex surface some whitish patches, in one place more yellowish. Brain preserved unopened, but right ventricle punctured and nasgar tube inoculated with point of needle.

Abdominal cavity contains no fluid. Peritoneum of normal aspect. Abdominal organs normally situated.

Spleen 13 × 11 × 2½ cms. Capsule smooth, transparent. Colour, structure, and consistence normal.

Kidneys 12 × 6½ × 3 cms. Capsule easily detached; Surface grey with a few dilated stellate veins. Cortex grey, with some yellowish patches. Pyramidal bases hyperaemic. Consistence normal.

Liver 30 × 15 × 7 cms. Surface yellowish grey, with hyperaemic patches. Capsule smooth, transparent. Cut surface uniformly yellowish grey. Consistence slightly diminished, pasty.

Pancreas of normal size and consistence, slightly hyperaemic.

The *stomach* contains a considerable amount of greenish fluid, and, adherent to the mucosa, black, coffee-ground like, mucous matter, with numerous blood-streaks. The mucosa shows considerable hyperaemia, chiefly of the posterior wall, and numerous petechiae, especially on both sides of the lesser curvature, and on the posterior wall. The duodenum contains greenish black fluid, and its mucosa is slightly hyperaemic. In the middle portion of the jejunum there is a small ecchymosis, and in the remaining portions of the bowel some hyperaemic patches are observed.

The *bladder* contains about 100 c.c. yellow, slightly turbid urine. Mucosa normal.

No liquid in *pleurae;* but adhesions over the superior lobe of the left lung.

The *lungs* show intense hyperaemia of postero-inferior portions; no oedema, no infiltrations, no infarcts. No definite signs of tuberculosis in lungs, or bronchial lymph-nodules.

Mucosa of trachea hyperaemic.

Thyroid body of normal aspect.

Pericardium contains no excess of fluid. No petechiae.

Pulmonary artery free.

Endocard and myocard of normal aspect.

Urine: Slightly turbid, acid, contains albumin; no sugar; faint reaction for bile-pigments; numerous hyaline and granular casts; a few leucocytes and erythrocytes; numerous epithelial cells.

Anatomical Diagnoses: Gastritis ecchymotica. Metamorphosis adiposa hepatis m.g. Pancreatitis parenchymatosa acuta. Nephritis parenchymatosa acuta. Hypostasis pulmonum m.g. Leptomeningitis cerebralis veteris dati l.g. Icterus universalis l.g.

Histological examination:—

Liver: Extremely intense vacuolization of cells (fat), as a rule most marked around portal vessels. Marked necrobiotic changes which are often most intense in the places of advanced fat changes around the portal vessels, but in other parts most intense in the intermediate zone. Considerable capillary hyperaemia, irregularly distributed. Some microcellular infiltration of the periportal connective tissue.

Pancreas: Hyperaemia and slight haemorrhagic infiltration; oedema of stroma. Marked necrobiotic changes, the cells in Langerhans's islets being as seriously affected as those in the parenchyma proper.

Kidney: Vacuolization of basal portions of cells (fat). Some increase of stroma which is oedematous; slight microcellular infiltration, considerable hyperaemia. Coagulated serum in Bowman's capsules around the retracted glomeruli.

Microbiological examination :—

Nasgar tubes, *spinal fluid :* Sterile. Nasgar tubes, ventricular fluid, Gram-negative bacteria, no diplococci. In smears of peripheral blood a few intracellular ring-shaped organisms are found of the type of *Paraplasma flavigenum.*

No protozoal organisms are found in smears of organs, but in smears from spleen and liver various bacteria are observed ; amongst these are diplococci which are Gram-negative and in several instances intracellular.

Case 36. P.B., 11 years, black, Tollgate, with Dr. Earle, May Pen. Living in the same house as No. 35.

The boy had been in poor health previously and often had ' fever.' He was taken ill on Tuesday, February 4, 1913, at about 3 p.m., when he began to vomit ; he vomited all night and during the next day, until he died near midnight on the 5th, without medical attendance. He had no convulsions, but retraction of the head was observed by the mother. During his previous illness he had never suffered from vomiting.

Post-mortem examination : At 8 p.m., on February 6, 20 hours after death. Poorly nourished body. Rigor present. No jaundice. No cutaneous affections or parasites.

No *spinal fluid* obtained by lumbar puncture ; spinal cord not examined. Dura mater cerebralis normal. Pia mater shows slight acute hyperaemia over all parts of the brain. No oedema or exudation. No distension of lateral ventricles ; a small quantity of clear fluid is seen at the basis of the skull. Brain substance shows normal aspect.

The *abdominal cavity* contains no fluid. The peritoneum is of normal aspect. The abdominal organs are normally situated.

The *mesenteric lymph nodules* are considerably enlarged and hyperaemic, of normal consistence.

The *gastric and intestinal vessels* are considerably distended. There are a few easily detached intussusceptions of the small bowel. Tongue coated with white fur. No haemorrhage of gums. Tonsils slightly enlarged ; no signs of acute inflammation. Oesophagus normal. The stomach contains brownish, apparently haemorrhagic fluid ; the mucosa is hyperaemic and shows on a small portion of the posterior surface, close to the lesser curvature numerous petechiae. The duodenum is hyperaemic, otherwise normal ; jejunum and ileum, show hyperaemic patches ; no haemorrhages, and no swelling of Peyer's patches, or solitary follicles ; no ulcers. No intestinal parasites.

Liver : 22 × 13 × 6 cms. Capsule smooth, transparent ; colour pale grey with yellowish patches ; consistence distinctly diminished. The duodenal papilla presents a small, prominent mucous mass which, however, gives way when pressure is exerted on the gall bladder, and the bile then flows out freely ; mucosa of bile ducts and gall bladder normal ; bile remarkably dark, and somewhat viscid.

Pancreas is hyperaemic, and of considerably diminished consistence.

Several *lymph nodules* at the porta hepatis are enlarged and show haemorrhagic infiltration.

Spleen : 19 × 5 × 1½ cms. Capsule smooth, slightly thickened ; tissue somewhat pale, with slightly prominent follicles.

Suprarenal capsules normal.

Left *kidney* 8 × 5 × 2 cms. Capsule easily detached ; surface is smooth, shows capillary hyperaemia, but no distension of stellate veins ; cortex pale yellowish ; pyramidal bases considerably hyperaemic. The right kidney presents the same characters as the left. Mucosa of renal pelves, ureters, and bladder normal.

The *pleurae* contain no fluid and show no adhesions. The lungs are pale, showing only a very slight hyperaemia of the posterior portions; the lung tissue contains air throughout; there are neither oedema, infiltrations, nor infarcts.

The *pericardium* contains a few drops of clear fluid. The visceral pericardium shows a considerable number of minute petechiae and small ecchymoses on the anterior, lateral, and posterior surface of the left ventricle, especially close to the auricle, and also a few similar ones on the auricle. Heart conus $7 \times 7 \times 2\frac{1}{2}$ cms. Endocard and valves normal. Myocard pale grey, with more yellowish patches; consistence distinctly diminished.

The *larynx* and *trachea* are normal; the smaller bronchi contain mucous fluid.

The *thyroid body* and the *hypophysis cerebri* are of normal size and aspect.

Anatomical diagnoses: Gastritis ecchymotica. Hyperaemia intestinorum. Metamorphosis adiposa hepatis. Pancreatitis parenchymatosa acuta. Nephritis parenchymatosa acuta. Lympho-adenitis mesenterialis et portae hepatis. Perisplenitis chronica l.g. Ecchymoses pericardii. Metamorphosis adiposa myocardii. Hypostasis pulmonum l.g.

Histological examination:—

Spleen: Hyperaemia; oedema of stroma.

Pancreas: Localized necrobiosis; oedema of stroma.

Kidney: Localized hyperaemia, granular change and karyolysis in many cells in the convoluted tubules. Some basal vacuolization.

Stomach: Some increase of lymphoid tissue and moderate diffuse microcellular infiltration; oedema of submucosa.

Duodenum: Hyperplasia of Brunner's glands and lymphoid tissue, slight microcellular infiltration. Necrobiotic changes in the mucosa.

Brain: Hyperaemia and slight superficial microcellular infiltration.

Case 38. D.L., 8 years, black, admitted to Kingston Hospital in a dying condition, on February 7.

The boy had been ill with fever and vomiting. No further particulars were obtained.

Post-mortem examination, on February 7, at 12.30 p.m.

Anatomical diagnoses: Icterus universalis l.g. Gastritis ecchymotica c. erosionibus. Metamorphosis adiposa hepatis. Nephritis parenchymatosa acuta c. metamorphosi adiposa. Metamorphosis adiposa myocardii. Hyperaemia piae matris cerebralis. Pancreatitis parenchymatosa acuta.

Black and blood-stained mucus is adherent to the wall of the stomach, which contains a few milk-clots.

A few slightly hyperaemic patches are seen in the small and large bowel. The Peyer's patches are likewise slightly hyperaemic.

Liver: 650 grms., yellowish grey, homogeneous, pasty.

Kidneys of normal size; capsule easily detached; surface congested, with yellow patches; cortex yellowish; consistence diminished.

Spleen of normal aspect. Slight diffuse hyperaemia of pia cerebralis, no odema or inflammatory exudate.

Urine: The bladder contains a considerable quantity of pale urine, which contains a small quantity of albumen, no sugar, no bile-pigments, few hyaline casts, numerous epithelial cells, a few erythrocytes and leucocytes.

Histological examination:

Lymph nodules: Hyperaemia and perinodular lymphoid infiltration.

Myocard: Numerous small vacuoles (fat) in muscle fibres.

Liver: Advanced fatty metamorphoses, all zones being equally affected. Marked anaemia.

Pancreas: Advanced necrobiotic changes, most nuclei being invisible and cell limits poorly defined.

Kidney: Large vacuoles (fat) occupying basal parts of cells in convoluted tubules. Most nuclei staining well, straight tubules not affected. Marked anaemia.

Medulla: Oedema and slight infiltration of pia.

Case 47. A.W., 20 years, black, Windward Road, Doncaster Pen. Seen in Public Hospital, Kingston, with Dr. Thomson.

Admitted in a weak condition. History of sudden onset of vomiting last night. No history of previous illness. Her infant, $1\frac{3}{4}$ years old, mentioned in note to case 48, was attacked earlier in the evening, and died. A nephew also started vomiting during the night (case 48).

Temperature 98·4° on admission. Pulse small and irregular. Patient complains of pain in the epigastrium.

Temperature went up in the afternoon to 99° and 100°, but in the evening again down to 98°, where it remained until the time of death. Heart's action was in the afternoon regular; sounds clear. Lungs clear. Tenderness in epigastrium. Spleen not felt. Pupils equal, not contracted, nor dilated, react to light and accommodation. No vomiting since admission, but drowsiness. No rigidity; Kernig's symptom not present.

February 19. During the afternoon patient's condition grew weaker; the drowsiness passed into coma. The breathing became laboured; the pulse was very small and irregular, but responded readily to stimulation. No rigidity of limbs.

At 4 a.m., patient appeared to be sinking; she was quite comatose, with Cheyne-Stokes's respiration; pulse small and irregular.

Lumbar puncture gave clear, watery fluid. Urine could not be collected.

Death at 5.35 a.m.

Post-mortem examination at 7 p.m., on February 19, 13 hours after death.

Body well nourished; rigor present; no jaundice. A few *Pediculi capitis*, no other parasites, nor affections of the skin.

Axillary *lymph nodules* moderately enlarged and hyperaemic; inguinal enlarged, cervical normal.

The subcutaneous fat shows, in various places, small haemorrhages.

Dura spinalis normal; pia hyperaemic, especially in lower part. Spinal cord normal. Cerebral dura normal; pia considerably hyperaemic, particularly on the convex surface. No exudation, but some haziness over the basis and over the Sylvian fissures. Lateral ventricles not distended, contain a few drops of clear fluid. Brain substance normal.

Hypophysis slightly enlarged, hyperaemic, and with soft, yellowish patches.

Pleurae normal. Lungs slightly hyperaemic in postero-inferior parts, contain a few minute haemorrhagic infarcts. Smaller and larger bronchi, trachea and larynx show diffuse hyperaemia and some mucous secretion. Bronchial lymph nodules not enlarged.

Pericardium contains a few c.c. of serous fluid; conus 9 × 11 × 5 cms.; epicard shows several small ecchymoses on posterior and left surface of left ventricle and auricle. Endocard and valves normal. Myocard shows small yellowish patches; consistence somewhat diminished; left ventricle fairly well contracted.

Thyroid body normal.

Peritoneum normal, without fluid. Abdominal organs normally situated.

Spleen : Small, 11 × 5 × 2 cms. Capsule normal; colour pale; follicles grey, slightly prominent.

Suprarenal capsules normal.

Mesenteric *lymph nodules* slightly enlarged, not hyperaemic.

Left *kidney :* 12 × 6 × 4. Capsule easily detached; surface extremely pale except in a few places where there is some dilatation of the stellate veins. Cortex pale, yellowish grey, and some of the septa completely yellow, of almost caseous aspect, the parenchyma structureless, bulging on the cut surface. Pyramids pale. Consistence of the whole organ considerably diminished, and of the yellow parts, soft, pasty. Right kidney 11 × 7 × 3 cms.; characters similar to those of the left. Mucosae of renal pelves, ureters and bladder normal. The bladder contains a small quantity of pale urine.

Liver : 29 × 15 × 8 cms. Capsule normal; surface pale, yellowish; tissue yellowish grey, homogeneous; consistence pasty.

Pancreas of normal size, but of somewhat diminished consistence.

Tongue covered with white fur. Tonsils normal.

The *stomach* contains a large quantity of reddish black fluid, with black particles, coffee-grounds like, also some blackish mucus (the fluid is acid, and gives reactions for haemoglobin); mucosa hyperaemic; no haemorrhages visible. Duodenum contains considerable quantity of dark, sanguinolent fluid, mucosa intensely hyperaemic, with minute petechiae. Jejunum normal. Ileum hyperaemic. Colon shows marked hyperaemia. Appendix 18 cms. Two ascarides in ileum.

Anatomical diagnoses : Hyperaemia meningum. Hypostasis et infarcti haemorrhagici parvi pulmonum. Ecchymoses pericardii. Metamorphosis adiposa myocardii. Ecchymoses telae adiposae subcutaneae. Gastritis acuta haemorrhagica. Duodenitis acuta haemorrhagica. Hyperaemia coli. Metamorphosis adiposa hepatis maximo gradu. Nephritis acuta duplex parenchymatosa c. metamorphosi adiposa m.g. Metamorphosis adiposa (?) pancreatis. Lymphadenitis axillaris, inguinalis et mesenterialis acuta. Pediculi capitis. Helminthiasis intestinalis.

Histological examination :—

Axillary lymph nodules : Hyperaemia.

Pituitary body : Hyperaemia.

Suprarenal capsule : Diffuse hyperaemia, especially intense in the periglandular tissues where also infiltrating haemorrhages are observed.

Liver : Intense fatty metamorphosis. Necrobiotic changes are present throughout the organ; in some places these phenomena show the Rocha-Lima type, but most often only insignificant remains of protoplasm are preserved in the cells surrounding the hepatic veins, where the affection in many places appears mostly advanced. There is slight microcellular infiltration of the periportal tissues.

Pancreas : Minute fat droplets are observed in the alveolar epithelia and slightly larger droplets in the cells of Langerhans's islets.

Kidney : The epithelia of the convoluted tubules show granulation of protoplasm and their basal portions are occupied by vacuoles which correspond to fat droplets in osmic acid specimens. These phenomena are present with some irregularity in nearly all the convoluted tubules and exceedingly intense in many of those of the first order. They are also very intense in the ascending limbs of Henle's loops, but there is hardly any fatty change of the cells in the descending limbs and collecting tubules. A number of patches of intense capillary hyperaemia

are observed and in the neighbourhood of these patches there is some microcellular infiltration. There is moderate increase of the stroma especially of the pyramids, the glomeruli are slightly retracted and minute fat droplets are observed in their cells.

Myocard : Moderate fatty change.

Microbiological examination :—

Spinal fluid, nasgar tubes : Gram-negative diplococci which change glucose, galactose and maltose, and saccharose slightly, but not lactose and mannite ; also some colonies of Gram-negative cocco-bacilli.

Heart blood, nasgar tubes : Gram-negative cocco-bacilli. Smears of peripheral blood and heart blood show no protozoa but in the heart blood some large bacteria.

No organisms are seen in smears from spinal fluid after centrifugalization.

Case 54. D.A., 7 years, coloured, Eastward Villa, Hagley Park. Seen, after death, with Dr. C. R. Edwards, Halfway Tree, on February 25 and 26, 1913.

The child has never been ill before. She felt slightly unwell on February 23, in the evening, was crying and complaining of pain in the stomach ; during the following night she woke up, at 1.30 a.m., February 24, groaning, and had three attacks of convulsions with clonic spasms. During the next day she vomited several times clear fluid, and did not take any food ; she did not seem to have fever, but, according to the mother, the skin turned yellow (vide infra). She was awake during the whole of the following night, restless, semi-unconscious, and complaining of abdominal pain. At 9 a.m., on February 25, she was brought to Dr. Edwards' office, where she remained until her death. Whilst here, several (8) convulsions were observed ; they consisted of clonic spasms, with final tonic contractions ; she vomited repeatedly, to begin with clear, watery fluid, but later on dark, coffee-grounds like matter, and reddish, thin fluid, with suspended small particles, in large quantities. She was unconscious, became gradually weaker, and died at about 4 p.m., just a quarter of an hour before my arrival.

When seen after death, the body appeared rather yellow, but there was no trace of yellow in the conjunctivae. Spinal fluid, blood, urine and vomited matter collected by Dr. Edwards.

Spinal fluid : About 20 c.c., clear.

Bladder contained only a few drops of pale urine.

Post-mortem examination, at 11 a.m., on February 26, 19 hours after death :—

Body somewhat poorly nourished. Rigor present. Extensive livid patches on the back, invading even the flanks. No jaundice of conjunctivae, but the body has a peculiar yellow colour. No skin affection, and no external parasites, except lice in the hair.

Spinal venous plexus gorged with blood. Dura spinalis normal, cerebralis slightly hyperaemic ; pia spinalis and cerebralis intensely hyperaemic, and the latter slightly oedematous at the basis. Lateral ventricles not distended, contain no fluid. Brain and cord normal.

Hypophysis softened, otherwise normal.

Inguinal, axillary and cervical lymph nodules considerably enlarged and intensely hyperaemic.

Left *kidney :* 8 × 5 × 2 cms. Capsule easily detached ; surface smooth, dark, with capillary hyperaemia, shows a few yellow patches ; cortex reddish grey ; glomeruli normal ; columnae Bertini pale, yellowish ; pyramidal bases hyperaemic ; consistence considerably diminished. Right kidney shows same characters as left.

Tonsils : Hyperaemic, contain several small pus foci.

Mucosa of pharynx swollen and hyperaemic.

Mesenteric lymph nodules considerably enlarged, not hyperaemic.

The *stomach* contains a considerable quantity of greenish black fluid, with minute, brownish black particles; blackish mucus is adherent to the mucosa, which shows intense hyperaemia of cardiac portion of anterior wall, and numerous petechiae. On the posterior wall are several small erosions, without any sign of haemorrhage.

Duodenum: slightly hyperaemic. Jejunum shows patches of hyperaemia; ileum pale, colon shows several hyperaemic patches.

Liver: 21 × 14 × 5 cms. Capsule normal; colour reddish grey, with yellow patches; substance yellowish grey, with patches of pronouncedly yellow colour, and a few patches of hyperaemia; consistence considerably diminished, and somewhat friable.

Pancreas: Slightly enlarged; the caudal portion shows intense hyperaemia, and some haemorrhage into the tissue; caput and corpus pale; the whole organ is of considerably diminished consistence.

Anatomical diagnoses: Hyperaemia meningum. Poly-lymphadenitis acuta. Metamorphosis adiposa myocardii, et hepatis. Pancreatitis acuta. Nephritis acuta incipiens duplex. Hypostasis pulmonum. Gastritis acuta ecchymotica. Hyperaemia jejuni and coli. Elytritis. Endometritis. Cystitis? Helminthiasis intestinalis. Tonsillitis acuta. Pharyngitis acuta.

Histological examination:—

Axillary lymph nodules: Slight diffuse hyperaemia, in some places more pronounced. One considerable haemorrhage and several smaller ones.

Myocard: Fragmentation, numerous small fat droplets in fibres.

Liver: Diffuse hyperaemia, one or a few large vacuoles (fat) in nearly every cell. Slight microcellular infiltration of periportal tissue and penetrating into the lobules.

Pancreas: Advanced necrobiotic changes.

Kidney: Numerous fairly small fat droplets in the epithelial cells near the basal membrane, mostly in the convoluted tubules, less in the straight tubules and only very few cells in the glomeruli.

The *spinal fluid* is clear, alkaline, gives fairly intense albumin reaction. The centrifugalized deposit consists chiefly of erythrocytes; also some lymphocytes and a few polymorphonuclear leucocytes are seen.

Microbiological examination:—

Peripheral blood: No parasites. *Heart blood*, nasgar tubes, long Gram-negative bacteria. *Spinal fluid*, nasgar tubes, short thick Gram-negative bacteria which change all the sugars tested. *Vomits:* The first portion examined is clear bile acid; second portion is reddish black, with suspended dark brown particles. It is intensely acid and contains erythrocytes (microscopical) and haemoglobin (Guajac test).

Group G contains four cases: Nos. 6, 26, 45 and 58. Case 6 was a typical case of infantile gastro-intestinal atrophia, and, when I saw the case, there was no suspicion whatever of 'vomiting sickness.' My attention was, however, drawn to this case, because it was said to have been diagnosed as 'vomiting sickness' about three weeks previously. I regard this as an example of mistaken diagnosis, which is apt to occur, especially in recovering cases. This case

I exclude absolutely from the discussion as far as 'vomiting sickness' is concerned, but it may be mentioned that even in this case meningeal hyperaemia was present, contrasting with the marked anaemia of all other organs. Case 26 was also one of general atrophia, but this time in an old man. In this case Gram-negative diplococci were cultivated from the spinal fluid, a result which there is no reason to consider otherwise than as an accidental post-mortem infection. Case 45 resembled the type of 'vomiting sickness,' but the presence of numerous malarial parasites affords sufficient explanation of the symptoms and of the anatomical lesions. This case serves to illustrate the difficulties of diagnosis and is therefore given in full. Malarial parasites were also found in Case 58, but in small numbers; the diagnosis of malaria as *causa efficiens* must therefore be regarded with some doubt, and a diagnosis of 'vomiting sickness' has to be considered. The patient in Case 58 was a sister to the one is Case 57 belonging to Group B, as organisms of the meningococcus type were found. The two sisters were taken ill and died at the same time, obviously from the same disease; it seems, therefore, that the essentially different results of the microbiological examinations must be disregarded and both cases classified as 'vomiting sickness.'

Case 6. J.R., 26 months, Porus. Ill for three weeks, with fever, vomiting and diarrhoea with green stools; no convulsions, no shrieks.

Post-mortem diagnoses: Atrophia ventriculi et intestinorum. Anaemia universalis. Hyperaemia piae matris cerebralis. Hypostasis pulmonum.

Case 26. R.L.D., about 60 years, black, from Belvedere, Portland, not seen before death, but reported as having been taken ill acutely with vomiting. Later it was found out that he had been weak for several months and had vomited for some days before death. The corpse was brought down to the hospital mortuary and a post-mortem examination performed, with Dr. George.

The autopsy on February 1, 1913, showed simple atrophy of all organs and multiple cysts in both kidneys. There were no signs of cerebral meningitis and no affection of the stomach. The spinal cord was not examined, but lumbar puncture gave no fluid, except a single drop, with which a nasgar tube was inoculated.

Histological examination:—

Liver: Atrophia pigmentosa.

Microbiological examination:—

Nasgar tubes, *spinal fluid:* Gram-negative diplococci in groups which change glucose, galactose, saccharose and maltose, but not lactose.

Case 45. M.K., 8 years, black, Spanish Town Road. Kingston Public Hospital, February 15, 1913.

Admitted at 5 p.m., suffering from vomiting and convulsions, which had started the same day, suddenly. Head retracted. Death at 7.20 p.m.

Post-mortem examination, at 12 noon, on February 16, about 17 hours after death :—Body fairly well nourished. No skin affection. Hyperaemia of lips and gums ; small ulcers on lips. No jaundice. Rigor present.

Cervical and axillary *lymph nodules* enlarged and hyperaemic, inguinal enlarged.

Lumbar puncture gives only one or two drops of serous, slightly blood-stained fluid. Spinal dura normal, pia hyperaemic ; cord normal in dorsal portion, the only part examined. Cerebral dura slightly hyperaemic, pia considerably hyperaemic, especially on convex surface ; a few drops of serous fluid at basis ; no fibrinous exudation, one or two c.c. of clear fluid in lateral ventricles ; brain substance normal, slightly pale.

Hypophysis small, 6 × 4 × 3 mms., normal in appearance.

Pleurae normal. Lungs show slight hyperaemia, most marked in postero-inferior parts. Bronchi hyperaemic, contain some mucous secretion ; trachea and larynx normal. Bronchial lymph nodules slightly enlarged and anthracotic.

Pericardium contains no fluid ; epicardium, endocardium and valves normal. Heart conus 6 × 7 × 2½ cms. ; left ventricle fairly well contracted. Myocardium normal. Pulmonary artery and aorta normal.

Thymus 6 × 4 × 1½ cms., normal, in appearance ; consistence slightly increased.

Thyroid body normal.

Peritoneum normal without fluid. Abdominal organs normally situated.

Spleen : 15 × 9 × 5 cms. Capsule slightly folded and thickened ; substance homogeneous dark purplish grey, without prominent follicles. Consistence considerably diminished.

Mesenteric lymph nodules slightly enlarged and hyperaemic.

Suprarenal capsules : Considerably hyperaemic, the left most pronouncedly so ; the left is also slightly enlarged and contains several small haemorrhages

Right *kidney :* 8 × 4½ × 2½ cms. Capsule easily detached ; surface smooth, with capillary hyperaemia ; some yellowish grey patches on surface ; cortex hyperaemic in superficial part, grey in columnar portion. Slight hyperaemia of pyramidal bases. Left kidney shows more marked hyperaemia.

Renal pelves, ureters and bladder slightly hyperaemic ; bladder contains about 30 c.c. of clear yellow urine.

Genital organs normal.

Liver : 25 × 16 × 5 cms. Capsule normal ; surface dark purple, with a few small yellowish patches ; structure and consistence normal. Bile ducts patent and normal ; gall bladder contains a considerable quantity of yellowish bile.

Pancreas normal in size ; slight hyperaemia ; considerably diminished consistence.

The *tongue's* entire surface is covered with a whitish fur. Left *tonsil* slightly enlarged, haemorrhagic infiltrated and contain some inspissated pus. Oesophagus normal. The *stomach* contains alimentary fluid and mucus. The mucosa shows a few hyperaemic patches, is otherwise normal. Duodenum shows hyperaemia and swelling of follicles. Jejunum shows patches of hyperaemia and slight follicular swelling in its upper part. The solitary follicles and Peyer's patches in the lower part of the ileum are swollen, but not hyperaemic. Appendix normal. Colon hyperaemic, with follicular swelling and dark, pigmented spots on follicles. No worms.

Anatomical diagnoses : Hyperaemia meningum, pulmonum, intestinorum et capsulae suprarenalis. Splenitis acuta in chronica pigmentosa. Metamorphosis adiposa l.g. Hepatis et pancreatis. Gastro-enteritis follicularis. Poly-lymphadenitis acuta.

Histological examination :—

Axillary lymph nodules : Moderate hyperaemia.

Tonsils : Intense hyperaemia.

Liver : Slight fatty change.

Pancreas : Slight localized necrobiotic changes.

Kidney : Advanced necrobiotic changes of the epithelium of the convoluted tubules with karyolysis and granulation of protoplasm. No vacuoles, and glomeruli normal. Epithelia of straight tubules well preserved. Moderate irregularly distributed hyperaemia.

Microbiological examination :—

Spinal fluid : Nasgar tubes remain sterile.

Heart blood, smears : *Plasmodium vivax ;* abundant infection with young parasites.

Spleen : smears ; *Plasmodium vivax :* abundant pigmentation.

Heart blood : Nasgar tubes remain sterile. I haemoglobin-agar growth of Gram-negative cocco-bacilli.

Case 58. D.M.M., 4 years, black, seen, after death, with Dr. Peck, Spanish Town, on March 5, 1913.

Post-mortem examination :—

Lumbar puncture gives clear fluid, with admixture of blood. Dura normal. Pia slightly hyperaemic on lumbar portion of cord and convex surface of brain ; normal at basis. Lateral ventricles normal. Brain and cord normal.

Cervical, axillary and inguinal lymph nodules, enlarged and slightly hyperaemic, especially the axillary.

The *stomach* contains alimentary fluid ; slight hyperaemia of mucosa.

Duodenum shows considerable follicular swelling. Jejunum normal, except for a few hyperaemic patches. Ileum normal. Appendix and colon normal. One *Ascaris lumbricoides.*

Anatomical diagnoses : Hyperaemia piae matris l.g. Lymphadenitis acuta l.g. Hyperaemia pulmonum. Petechiae epicardii l.g. Hyperaemia ventriculi l.g. Duodenitis follicularis. Nephritis acuta l.g. Helminthiasis intestinalis.

Urine : Acid ; contains no albumin, sugar, bile pigment, or casts.

Histological examination :—

Axillary glands : Considerable hyperaemia, increase of stroma.

Spleen : Pigmentation.

Liver : Irregular hyperaemia ; marked fatty change.

Pancreas : Moderate fatty change.

Kidney : Moderate fatty change, some increase of stroma and some microcellular infiltration. Epithelia well preserved.

Duodenum : Brunner's glands prominent. Microcellular infiltration and irregular hyperaemia.

Microbiological examination :—

Spinal fluid, nasgar tubes : remain sterile. Heart blood, nasgar tubes remain sterile. Heart blood smears : *Plasmodium vivax*, sparse infection.

Group H includes five cases, Nos. 3, 21, 22, 28 and 53, in which I was unable to obtain sufficient data to attempt a diagnosis. These cases may be left entirely out of the discussion.

V. DESCRIPTION OF VOMITING SICKNESS

It is quite obvious, from the preceding report of cases, that not all of those described are of the same nature. An attempt to determine the nature of 'vomiting sickness' may be preceded by a re-grouping of the cases in order to establish a well-defined field for discussion. So far, it is necessary to include amongst the chief characteristics the negative one, that a diagnosis of vomiting sickness is inadmissible as long as a case can be diagnosed otherwise on clinical and pathological grounds. The microbiological examination does not necessarily decide this matter. Two or more diseases may be caused by the same pathogenic agent and nevertheless differ so essentially from each other that they are in all systems classified under different headings.

TABLE B

Vomiting Sickness			Possibly Vomiting Sickness				Yellow Fever	Malaria	Infantile or Senile Atrophy
Group A	Group B	Group C	Group D	Group E	Of Group F	Of Group G	Of Group F	Of Group G	Of Group G
16	12	12	5	3	4	1	1 (or 5)	1	2
40 cases			13 cases				1 (or 5) cases	1 case	2 cases

Furuncles, osteomyelitis, and generalised sepsis are often due to one and the same organism, but it would obviously be wrong to consider them as identical diseases. Similarly, 'vomiting sickness' might be caused by the same germ that is responsible for meningitis, for instance, and still with some right claim a name as independent disease. From this point of view we shall have to consider only the cases in Groups A, B and C. Those in the other groups either do not correspond to the type represented in A, B and C, or they are insufficiently observed, or they can be otherwise diagnosed.

It appears that an arrangement like that given in Table B would represent a natural classification of the provisionally established groups, leaving out of consideration the non-defined group H.

The following clinical and pathological description applies to the cases which I would regard as 'vomiting sickness.'

Definition.—'Vomiting sickness' is an acute infectious disease which chiefly attacks children, often with fatal issue in a very short time, and which has so far only been observed in blacks, coloured persons and Hindoos in Jamaica.

Etiology.—The question of a specific pathogenic germ has not yet been fully solved; it is discussed in the following section. Deficient hyperaemic conditions, malnutrition and exposure to cold have been mentioned by various observers as etiological factors of importance, but there is no evidence that any of these factors should be very essential. Personally, I feel inclined to eliminate malnutrition entirely from this list, as in my experience a considerable number of cases have occurred in well nourished, even fat, and absolutely healthy-looking individuals. The mode of transmission is unknown, and such suggestions as have been made in this respect are based more upon analogy than upon actual observation.

Distribution and epidemiology.—'Vomiting sickness' is, as far as the majority of the cases are concerned, a disease of people inhabiting poor huts more or less closely surrounded by bush. This, however, is by no means a rule without exception; several cases occurred in well-built and cleanly kept houses in villages, or situated on dry ground, apart from the bush.

Cases were observed, in this as in earlier outbreaks, in considerable numbers in some districts, and were altogether absent in others. This circumstance is, however, not one of very great importance. Districts are large in Jamaica, and it is no easy matter to trace the connection between ten and twelve cases scattered over an area of nearly a hundred square miles. As a matter of fact, a connection could rarely be demonstrated with certainty. When several cases occurred in one and the same house, a connection might of course be presumed to exist between them, but in each instance one would sooner think of a common source of infection than of transmission of the infection from one case to another, because the cases as a rule occurred either simultaneously or in very rapid succession. My material includes various examples of almost simultaneous occurrence of vomiting sickness in two or more members of one and the same family, living in the same house, as

in the following group of cases: Nos. 14, 15 and 16; 17 and child not observed; 35 and 36; 47, 48, and infant; 57 and 58. On the other hand, there are even more numerous instances of the isolated occurrence of the disease in one member of a family, although several other children were living in the same house.

Symptomatology.—The duration of the incubation period is unknown, but there is some reason to believe that it is short, considering the hyperacute character of the disease. Nothing definite has been recorded about the occurrence of prodromal symptoms.

The illness usually sets in suddenly, often in the night, or early morning, though this is by no means a constant feature; in fact, it is not uncommon that the symptoms are first observed in the evening and that death takes place during the night. Vomiting is very often the first symptom; it is usually watery or consisting of alimentary fluid, at any rate to begin with, but may later on become bilious or perhaps even haemorrhagic. Vomiting is not a constant, but an extremely common symptom; out of the forty cases in the Groups A, B and C, its occurrence is mentioned twenty-four times, and its absence six times; in the remaining ten cases no definite information was obtained. The next symptom, as regards frequency, is that of convulsions, which are sometimes described as clonic, but in other cases as tonic contractions, lasting for several minutes; an exact description of this symptom is at present hardly possible, as most cases were not medically observed. For the same reason, it is extremely difficult to discuss the occurrence and frequency of other nervous symptoms, but it can confidently be stated that coma is exceedingly common and that a sudden collapse apparently occurs in some cases. Rigidity of the neck muscles was not frequently observed, nor was rigidity of other muscles. Kernig's symptom was only recorded twice in the cases of the first three groups, but both with regard to this and the two foregoing symptoms it is important to remember that very few patients came under medical observation at all or sufficiently early to make a thorough examination possible.

Of other symptoms than those of a nervous character little is to be said. The course of the illness is so rapid that most organic lesions do not become recognisable clinically. Febrile temperatures

are seldom observed, but it seems probable that subnormal temperatures occur, though there is little evidence of this. The vomiting is, perhaps, in the majority of cases of cerebral origin, but just as often one would believe it to be due to gastric irritation. The severe renal affection which was frequently observed at the post-mortem examination could hardly be expected to produce any clinical symptoms; it may be specially mentioned that abundant urine was often found at the autopsy and that the amount of albumin was usually very small. It would appear that the urine found had been excreted before the development of the disease, or, at any rate, of the kidney affection. The rapidity with which the illness reaches a fatal termination shows, approximately, an inverse ratio to the age of the patients; infants die sometimes less than an hour after the appearance of the first symptoms, while cases in adults may last for one or two days.

Pathological anatomy.—The most striking anatomical lesions are: enlargement and hyperaemia of the lymphatic nodules, petechiae on the surface of the heart and in the gastric mucosa, necrobiosis of the pancreas, liver and kidneys, especially of the pancreas, and fatty change of the same and other parenchymatous organs. There is often a marked hyperaemia of the spinal and cerebral pia, but in other cases it was very moderate, and in others limited to the cerebral pia alone. The intensity of the lesions differed very much, especially that of the fatty changes, which was most marked in the cases of one or two days' duration in adults, whilst it was seen in its initial stage in cases of extremely rapid development.

General pathology.—The pathological physiology ought to explain the most striking feature of the disease, namely, the rapid course, the high mortality and the haemorrhagic degeneration and necrobiotic phenomena just described.

All these phenomena are readily explained as the results of an acute poisoning which may be due to the products of a bacterial or protozoal organism. If the vomiting is considered of cerebral origin, only the occurrence of cerebral symptoms has to be explained. All these symptoms, vomiting, convulsions and coma, may be due to an affection of the meninges, and this affection need not be a true meningitis; they may just as well be the expression of a state of 'meningismus.'

Mortality and prognosis.—According to some observers (Tillman and others, *Annual Report*) a very large number of cases of 'vomiting sickness' terminate in recovery. If this is true then many other observers, including myself, have only seen, or at any rate, have only diagnosed severe cases. This is possible, but for reasons stated above I consider it more likely that a large number of minor ailments, essentially different from 'vomiting sickness,' have been included in the statistics of the first-named observers. However, this controversy cannot be decided at present, and it may therefore be mentioned that the mortality has variously been found as low as about 2 per cent., and as high as about 100 per cent. In my own material, considering only the forty cases in Groups A, B and C, with thirty deaths, the mortality is 75 per cent. If a marked case of 'vomiting sickness' is seen, a very guarded prognosis should be given; recovery may take place, but the situation is always very serious.

Diagnosis.—Until the etiology of 'vomiting sickness' is more fully known, the diagnosis in vivo and post mortem must rest exclusively upon the symptoms described above.

Treatment.—No specific treatment can be recommended with any hope of success. Antimeningococcic serum might be used when 'meningococci' are found, but it is doubtful whether its use would be justified in other cases. Moreover, as Scott (1913, 2) has pointed out, its general use out in the country districts encounters many difficulties of a practical nature. An anti-serum might, perhaps, be prepared with the specific diplococcus, if this organism should finally be proved to be the cause of vomiting sickness. Vaccine treatment would hardly find any indication in this hyperacute disease. Hot baths and stimulating injections (ether, camphor, strychnine, etc.) may be recommended, but without much hope of success, especially as there will usually be very little time for the application of therapeutic measures.

Prophylaxis.—This question will be discussed in Section VII.

VI. THE NATURE OF VOMITING SICKNESS

At the onset of this discussion it is natural to ask, whether diseases identical to 'vomiting sickness' occur outside of Jamaica. Very little material is available which bears directly on this question.

In 1905 the Government of Jamaica made enquiries, in a circular letter to the authorities of other West Indian Islands, with regard to the possible occurrence of 'vomiting sickness' in other parts of the West Indies. Only two of the answers are quoted by Ker in the Annual Report (1906), the others being in the negative. The one positive answer is from Finlay, who mentions an outbreak of 25 similar cases with 5 deaths, amongst soldiers, in Cuba: he suggests that the disease is cerebro-spinal meningitis, no particulars are given in the quotation. The other positive answer deals with a disease (in Haiti) which has obviously no essential resemblance to 'vomiting sickness.'

Branch (1906), who has no personal experience with 'vomiting sickness,' does not admit it as a morbid entity, and is of opinion that similar cases occur elsewhere in the West Indies, carrying different names, and representing in reality well-known diseases, especially malaria and helminthiasis.

With regard to this opinion I must remark that it is difficult without personal observation to realize what 'vomiting sickness' means. Before seeing cases, I quite naturally had the same idea that it simply was something else; but the particular experience of seeing one fatal case following another in children who a few hours previously were playing out and who hardly developed any symptoms before they died, appeared to me almost unique, and left quite a different impression upon me.

Thus, until further evidence may be brought forward, we may assume that 'vomiting sickness' is a local disease of Jamaica.

Since the publication of Potter's and Scott's reports no further papers have appeared on the subject. It will now be justifiable to circumscribe the discussion, dealing, in the first place, with yellow fever and meningitis only.

With regard to yellow fever, some circumstantial evidence is in favour of this diagnosis. Jamaica was in earlier times one of the principal foci of the yellow fever infection, a veritable hot-bed for yellow fever. Even in this century, in 1904, a small outbreak occurred in Port Royal, which was apparently of spontaneous origin; this outbreak and the question whether there is any evidence that isolated, autochthonous cases occur, is discussed by Potter (1912).

Considering the question from an epidemiological point of view there is obviously good reason for investigating very carefully any disease which might prove an atypical form of yellow fever, and which might thus not only establish the fact of yellow fever endemicity in Jamaica, but also throw considerable light on yellow fever epidemiology in general. On the other hand, epidemiological evidence itself is almost sufficient to destroy this hypothesis. Yellow fever in native children in endemic areas is probably always a fairly benign disease; at any rate, there is no reason to suspect that it should ever acquire such a malignant, fulminant type as that of 'vomiting sickness.' The occurrence of these cases amongst the native population only, without ever attacking foreigners, is also very much against the theory of yellow fever. It might be argued that anti-yellow fever campaigns had made transmission impossible in the towns and that yellow fever had therefore become a disease of the bush instead of a town-disease, but even out in the country non-immune foreigners travel, apparently without ever suffering from attacks of yellow fever. Besides, cases of 'vomiting sickness' occurred, in my experience, in Kingston itself, and were common in the neighbouring district of Lower St. Andrew, which is much frequented by tourists, and in which many Europeans have their permanent residences. The seasonal incidence also is almost destructive to the hypothesis of yellow fever. Yellow fever occurs, without any doubt, at any time of the year in endemic areas, but it has never been known to show the exceedingly abnormal feature of dying out or becoming very rare during the hot and rainy seasons, when mosquitos, according to local information, are abundant, whilst, on the other hand, it prevails during the cool and dry months, during which, in my experience, mosquitos in general are rare, and *S. fasciata* very rare. Thus epidemiological evidence is rather against identifying 'vomiting sickness' with yellow fever; this should, therefore, only be done if the clinical and pathological evidence were absolutely overwhelming. But this is by no means so. The classical clinical symptoms of fatal yellow fever—as fever, black vomit, jaundice and anuria—are almost constantly absent. They may be absent, one or all, in cases of yellow fever, but it is hardly conceivable that they should all be absent in practically all cases in large outbreaks.

Fulminant yellow fever has been described, but not of the type which is met with in 'vomiting sickness.' The anatomical lesions in many cases of 'vomiting sickness' are certainly closely similar to those observed in yellow fever, but, then, I have previously (1911, 1) maintained that the anatomical diagnosis of yellow fever is even more difficult than the clinical one. No single pathological phenomenon is pathognomonic in yellow fever, but the combined picture of clinical symptoms and anatomical lesions is nearly always so. This combined picture, however, has not been met with in 'vomiting sickness.' With regard to the most important lesions it may be emphasized that haemorrhagic gastritis was common, but by no means constant in 'vomiting sickness,' and rarely as marked as in yellow fever; the affections of kidney and especially liver differed in showing more marked fatty changes and less marked necrobiotic phenomena than in yellow fever, especially when the comparatively slowly developing cases of 'vomiting sickness' are considered, as they naturally must be very particularly, as one would expect to find in such cases the most typical lesions. The Rocha-Lima type (see note on p. 438) of liver affection has not been marked in any case of 'vomiting sickness,' and, as a rule, not even a similar type has been observed.

The most striking feature of all is, perhaps, that these abnormal epidemiological, clinical and pathological characters, if the disease really were yellow fever, should have repeated themselves year after year in an absolutely typical manner. This certainly is a very strong argument which tends to show that we have to do with a specific and typical disease.

As regards parasitological evidence, little can at present be said. I had very rarely the opportunity of examining good blood smears taken whilst the patient was alive. In no case of typical 'vomiting sickness,' however, was the *Paraplasma flavigenum* found. I shall not here discuss the importance of the parasitological evidence in yellow fever, but must refer to my previous publications (especially 1911, 2 and 1912). The one case (Case 23) in which *P. flavigenum* was found, but which was not typical 'vomiting sickness,' has been discussed in the preceding section and will again be referred to in Section VIII.

The other disease with which 'vomiting sickness' has been, at

least partially, identified is cerebro-spinal meningitis. The evidence is that meningococci have been found by Scott (1912, 1 and 2, 1913, 1 and 2) in the cerebro-spinal fluid in a considerable number of cases of 'vomiting sickness.' Scott himself is, however, in his latest paper less positive with regard to the essential importance of his results, as far as 'vomiting sickness' is concerned, than he was to begin with. At any rate, there is more evidence in favour of the identity of 'vomiting sickness' with meningitis than with any other disease, and the question must be most seriously considered.

In the first place the bacteriological findings must be discussed. I shall not discuss the results published by Scott, but limit my remarks to my own investigations, in which I was, as stated above, greatly assisted by Dr. Scott himself. We fully realized—at least it soon became evident—the great difficulty in obtaining an absolutely reliable bacteriological diagnosis of meningococci, a difficulty which is, I believe, greater in Jamaica and probably in other tropical countries than elsewhere, as I shall presently explain. We therefore resolved to use the agglutination test, and not being in possession of a meningococcus strain from another locality used a strain obtained in one of Dr. Scott's first cases for immunization purposes. Unfortunately, however, the time at my disposal was too short; in fact, I gave the last of the commenced series of injections to several rabbits undergoing immunization, just before I left Jamaica. Thus I had to rely upon the morphological and cultural characters, staining reactions, and sugar reactions of the diplococci found. Of these characteristics, only the sugar reactions need a few preliminary remarks, as we, of course, only considered a diagnosis of meningococcus when colonies developed on ascites-agar, consisting of coffee grain-shaped diplococci, which did not retain the colour in the Gram-process. As regards the production of acid in sugar-media, there is no perfect agreement even between the most recent authors on the subject. Busse (1910) and Kutscher (1912) adopt Lingelsheim's view that meningcocci change glucose and maltose, but not other sugars, whilst Netter and Debré (1911), though in a general way confirming the same experiences, state that no very severe rules should be laid down. Scott (1913, 2) isolated in his first cases a diplococcus

which changed both glucose, maltose and galactose, leaving other sugars unchanged. These cocci were otherwise typical, and they were found in cases of anatomically well-characterized meningitis. For this reason we adopted these characters as a preliminary basis for classification, and according to this classification the distinction is made between the Groups A and B of my cases. The twelve strains isolated in the cases belonging to Group B showed the following reactions:—

No.	Glucose	Maltose	Galactose	Lactose	Saccharose	Mannite
1 ...	+	+	+	–	–	–
7 ...	+	+	–	–	–	–
8 ...	+	+	+	–	–	–
12 ...	+	+	+	–	–	–
20 ...	+	+	–	–	–	–
30 ...	+	+	+	–	–	–
33 ...	+	+	+	–	–	–
43 ...	+	+	–	–	–	–
44 ...	+	+	+	–	–	–
50 ...	+	+	+	–	–	–
55 ...	+	+	+	–	–	–
57 ...	+	+	+	–	–	–

Thus according to the classical rule, only the strains in Cases 7, 20 and 43 should represent typical meningococci. These three cases showed no more pronounced meningeal lesions than the others. In Group D, cases of meningitis, anatomically well characterized, the following reactions were obtained.

No.	Glucose	Maltose	Galactose	Lactose	Saccharose	Mannite
4 ...	+	+	–	...	–	–
19 ...	+	+	+	–	–	–
29 ...	+	+	+	–	–	–
31 ...	+	+	+	–	–	–
39 ...	+	+	+	–	–	–

In view of these results in the cases in Group D it appeared natural to uphold the distinction as previously stated.

No further unusual characters were observed during the examination of the cultures in Jamaica, but they became evident on re-examination of the cultures in Liverpool, where I continued several strains in Professor Beattie's laboratory. Already the survival of the cultures was remarkable. Some of them, which I brought home myself, survived twenty-seven days until sub-cultured. Other cultures, which Dr. Scott sent me, lasted for over two weeks. The following strains were examined: Cases 1 and 4 (A and B) and two of Dr. Scott's cultures, Stock I and Stock II. The following reactions were obtained:—

No.	Glucose	Galactose	Lactose	Saccharose	Mannite
1 (B.D.) ...	+	−	−	−	−
4 (V.C.) A ...	+	−	−	−	−
4 (V.C.) B ...	+	+	+	+	−
Stock I ...	+	−	−	−	−
Stock II ...	+	+	+	+	−

I now asked Dr. Ledingham, of the Lister Institute, for an immune serum for agglutination purposes. Unfortunately no rabbit immune-serum was available, but I received some of the Institute's anti-meningococcus horse serum, which Dr. Arkwright informed me showed marked agglutinating power, having been tested with numerous meningococcus strains. I likewise received normal horse serum from the Lister Institute. The results were as follows (macroscopical methods used):—

No.	Antimeningococcic serum						Normal horse serum
	1–20	1–40	1–80	1–160	1–320	1–640	1–20
1 (B.D.) ...	+++	+++	+	−	−	−	+++
4 (V.C.) A ...	+++	+++	−	−	−	−	++
4 (V.C.) B ...	+++	+++	+++	++	++	+	+++
Stock I ...	+++	++	+	−	−	−	+++

Only the strain 4 (V.C.) B gave a marked reaction; this result is remarkable, as this same strain gave quite atypical sugar reactions. It is, however, stated by several authors that there is a marked 'group reaction' amongst the meningococci and allied species. As the numbers of sub-cultures increased it was also observed that their aspects gradually changed; the growth was more abundant; and the colour more saturated whitish, characters which were particularly marked in Strain I (B.D.). The morphological characters remained the same, but the staining was somewhat irregular; it was found that on repeated occasions several of the strains showed a remarkable resistance to the decolorization after Claudius.

Dr. J. A. Arkwright also examined one of these cultures [4 (V.C.) B] and kindly informed me that he had observed considerable differences from the usual meningococcus strains, especially a considerably higher resistance. He found that the strain produced acid in glucose, maltose, galactose (slight) and cane sugar, but not in lactose.

Having learned that these strains possessed a considerable degree of variability, it appears advisable to revise Group A, as in many of the cases therein included diplococci were found, which were not considered in the discussion on meningococci on account of differences in sugar reaction or other characters. The following table shows these observations:—

No.	Glucose	Maltose	Galactose	Lactose	Saccharose	Mannite	Remarks
9	+	−	−	−	−	−	Diplococci forming chains
34	+	+	+	+	+	+	
37	+	+	+	±	−	−	
42	+	+	+	+	+	+	
46	−	+	−	−	−	−	Diplococci in chains

The strain in 37 showed but little difference from those previously described; in fact, sub-cultures gave no reaction in lactose media, which would perhaps justify including this case in Group B. The strains 9 and 46 might be considered typical

meningococci (according to Abel (1911) only glucose or maltose should be changed by meningococci), were it not for the peculiarity that the diplococci appeared in well marked chains. In this respect it resembles a strain which I found in a case of purulent meningitis observed in Yucatán (1910), and in which the formation of chains was a salient feature.

No pathogenic power was found by subcutaneous, intravenous, intraperitoneal and intracardial inoculations of living cultures of several strains into guinea-pigs or rabbits. These experiments need therefore not be detailed.

Thus, the bacteriological examination did not solve the question in an indisputable manner. The nature of the diplococci observed is not beyond question; but one thing seems evident; the distinction between Groups A and B, cases of 'vomiting sickness' type without and with meningococci, which was already at first but poorly marked, has been still more weakened by the closer examination of the cultures.

From the bacteriological data it is impossible to say whether the organisms isolated have any pathogenic importance or not. The organisms are not typical meningococci, but it is quite possible that they may cause pathological phenomena. I have, so far, come to conclusions similar to those of Arkwright (1909), who says about several organisms examined, 'They apparently have no relationship to the Meningococcus of Weichselbaum. They may, however, be the causal organism in some cases of meningitis, or may play a secondary part.'

The question, if possible to decide at the present time, must be decided on clinical, anatomical and epidemiological grounds. Clinically the typical cases of 'vomiting sickness' show considerable resemblance to fulminant cases of cerebro-spinal meningitis. Such cases have been described by many authors and are mentioned in recent text books. Thus, Osler (1912) says, 'Malignant form: This fulminant or apoplectic type is found with variable frequency in epidemics. It may occur sporadically. The onset is sudden, usually with violent chills, headache, somnolence, spasms in the muscles, great depression, moderate elevation of temperature, and feeble pulse, which may fall to fifty or sixty in the minute. Usually a purpuric rash develops. In a Philadelphia case, in 1888,

a young girl, apparently quite well, died within twenty hours of this form. There are cases on record in which death has occurred within a shorter time. Stille tells of a child of five years, in whom death occurred after an illness of ten hours; and refers to a case reported by Gordon, in which the entire duration of the illness was only five hours. Two of Vieusseux's cases died within twenty-four hours.'

'In malignant cases there may be no characteristic changes, the brain and spinal cord showing only extreme congestion, which was the lesion described by Vieusseux.'

Koplik (1907) mentions a malignant form of meningitis which is fatal within twelve, twenty-four or thirty-six hours. 'An adult or a child in apparent health complains of slight headache, there is nausea followed by vomiting, fever and unconsciousness in rapid succession. Death occurs in a short time, consciousness not being regained.'

A good description is also given by Ormerod (1905). This hyperacute form is particularly common in children, and is described in pediatric text books, for instance, Cautley (1910), Thiemich (1910) and Holt (1913). The latter says 'Cases of this kind are rarely seen except in an epidemic, and usually occur at its height. The onset is very abrupt, the course short and intense, and death may take place in from twelve to thirty-six hours. The following case illustrates this type: A little girl of ten years was well enough at 2 p.m. to carry a bundle of cloths a dozen city blocks. Returning home, she complained of intense headache, vomited frequently, and was so weak that she was obliged to go to bed. In a few hours she passed into deep coma, with very high fever and died at 11 p.m.' (p. 704.)

'There may be only a serous exudation and intense hyperaemia, which is doubtless much less marked after death than during life. The cerebro-spinal fluid is turbid and much increased in amount.'

The absence of definite inflammatory lesions of the meninges is recognized by the above authors and especially described by Busse (1910) and others. The whole question is particularly well dealt with by Netter and Debré (1911). Thus there is no doubt that such cases have been described, and so far there is no objection why the cases should not be diagnosed as hyperacute forms of meningitis.

With regard to the anatomical lesions of other organs than the meninges it may be mentioned that Busse (1910) lays stress upon the affection of the lymph-nodules, both peripheral and mesenteric. This corresponds to the conditions met with in vomiting sickness. On the other hand, such severe affections of liver, pancreas, and kidneys, as those found in my cases, have, as far as I know, not been described in cases of meningitis.

The typical case of 'vomiting sickness' corresponds fairly well to descriptions given of hyperacute cases of meningitis. It differs, however, as far as our present knowledge goes, in one important particular, namely, in the absence of fever. In the fulminant form of meningitis it is the rule that the temperature rises up rapidly, often to about 105°, or even more, whilst it is only mentioned as quite an exceptional thing that the temperature occasionally may remain normal until the fatal termination.

The epidemiological conditions, however, must also be considered. With regard to this question, practically all authors state that fulminant cases occur incidentally, isolated during epidemic outbreaks, and it is said by several, that they occur chiefly at the height of epidemics. The conditions in Jamaica are quite different. Here we have recurrent yearly outbreaks, and, perhaps, smouldering endemic prevalence.

It seems quite evident that no similar outbreaks of meningitis have ever been described. I therefore cannot accept at the present state of the investigations, the explanation, which at first sight seems the most probable, that 'vomiting sickness' is simply a form of meningitis. Experience has shown in several diseases that a germ which has for some time been regarded as a pathogenic one, has, on close investigation, been reduced to a secondary position as representing only a complicating infection. Further investigation is therefore absolutely imperative before the problem can be considered finally solved. It is quite possible that 'vomiting sickness' may be due to a blood-inhabiting, presumably protozoal, organism, and that a diplococcus infection of the meninges occurs as a frequent complication, as a rule not giving rise to any marked anatomical lesions, because of the rapidly following death, but in a few cases producing a typical fibrino-purulent meningitis. Another possibility is that the causal organism may be parasitic in

the intestine and produce exceedingly active toxins. Both explanations would correspond equally well to the clinical symptoms and both might also explain the anatomical lesions. The similarity of the latter to those observed in yellow fever makes one consider the existence of a blood parasite as very probable, but the marked affection of the lymph nodules in various parts of the body would, perhaps, be more in favour of a bacterial infection. In either case, whether the diplococci observed in these cases are the essential causal organisms or only of secondary importance, it seems, at any rate, very unlikely that they should be of no pathogenic importance at all. Whether these diplococci represent one or more species, it is impossible to say. At any rate they differ obviously from meningococci, gonococci, *Diplococcus crassus* and other known diplococci. The existence of more than one species is suggested by the very considerable differences in sugar reactions and agglutinating power. On the other hand, the great variability observed in some of the strains makes it possible to assume, until further investigation, the existence of only one new species. For this new species, which is very likely to be only a provisional one, I would suggest the name *Diplococcus jamaicensis*.

The chief characteristics of this species are that it is a diplococcus morphologically similar to gonococcus and meningococcus, as a rule Gram-negative, but occasionally decolourizing with some difficulty, showing fairly abundant growth on nasgar, the cultures being more resistant than meningococcus cultures, and changing at least glucose or maltose, but, as a rule, often one or several other sugars.

The mode of transmission of 'vomiting sickness' I have had no opportunity of investigating. As far as possible I examined the prevalence of mosquitos, ticks and other insects in houses which I visited with the object of seeing cases. Mosquitos were not commonly found, and when found were not abundant. Some were *Stegomyia fasciata*, some *Culex sp.*

I have not examined systematically cultures from the nasopharynx of contacts with 'vomiting sickness' cases, and I do not anticipate that very definite results can be obtained in this way until the etiology of 'vomiting sickness' is definitely settled. At present one would be in great difficulty with regard to a diagnosis between atypical strains of *Diplococcus jamaicensis* and

D. catarrhalis, and, further, one would not know how much importance should be attached to the positive results of the examination for *Diplococcus jamaicensis*.

Other theories, such as vomiting sickness being identical with malaria, with some form of helminthiasis, or with some kind of poisoning, find no support whatever in my observations.

VII. PROPHYLACTIC MEASURES

The absolute lack of knowledge about the mode of transmission of 'vomiting sickness' makes it impossible to lay down any rules with regard to prophylactic measures. On general hygienic principles it may be recommended that the poverty should be relieved and popular education, especially with regard to cleanliness, promoted. It is, of course, also important that anti-mosquito campaigns should be carried out, as well as campaigns against other insects, but to none of these measures can any specific importance be attached. It has been stated that no evidence points to insect transmission of the disease, and with regard to poor hygienic conditions it has also been stated previously that a considerable number of the individuals attacked by 'vomiting sickness' were absolutely healthy looking and well nourished; sometimes the houses and huts visited were also well kept and clean.

VIII. OTHER OBSERVATIONS IN JAMAICA

The one observation of a yellow fever case which has been mentioned in Section IV, would apparently tend to show that other cases might be of the same nature. There is, however, no evidence which makes it necessary to adopt this view. The cases occurred at the same time and in the same place as several cases of 'vomiting sickness,' but it showed a clinical course different from those cases, and the anatomical lesions were also different. There is, of course, no reason why the two diseases should not occur at the same time and in the same place, supposing that both exist in the Island. The problem is how to explain the occurrence of one

isolated case of yellow fever. This, however, is only what happens in many endemically but mildly infected places where the existence of yellow fever only becomes known when there is a considerable influx of foreigners. That outbreaks of yellow fever do not occur in Jamaica, though the disease may exist amongst the natives, is easily explained, I believe, by the relative freedom from mosquitos which the larger towns enjoy. The principal travelling public, the tourists, visit Jamaica during the winter months only, and at this time of the year I can safely assert that *Stegomyia fasciata* is not common in parts of Jamaica which I have visited, and which I believe in their natural conditions correspond fairly well with the various places which foreigners would visit.

I shall not mention here the laboratory observations on other subjects which were made during the 'vomiting sickness' investigation, but I want briefly to refer to a case which presented considerable interest, especially when considered in connection with my observations on Cutaneous Leishmaniasis in Yucatán (1912). This was a case of a fistulous and ulcerated tumefaction of the lower part of the femur, in which case I suspected infection with *Leishmania tropica*, but this parasite was never found, though repeatedly examined for. The particularly interesting point is that the exudation contained numerous intracellular diplococci, similar to those which were a constant feature in the cases of ear ulcer in Yucatán. Cultures were not obtained of this diplococci because of abundant mixed infection.

IX. OTHER PLACES VISITED

On my way to Jamaica and back I had the opportunity of visiting Trinidad, Barbados, the Panama Canal Zone, and Colombia. I visited the hospitals and saw interesting cases in Barbados and Trinadad and was fortunate in having a very good opportunity of seeing the sanitary arrangements on the Panama Canal Zone.

I also had the opportunity of discussing sanitary matters with Colonel Gorgas, Dr. Perry and other Officers of the Health Department, and was much impressed by the thoroughly well-established organization.

X. ACKNOWLEDGMENTS

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TABLE I.—SUMMARY OF CASES OBSERVED IN JAMAICA, PRINCIPALLY

Case Number	Initials	Date	Age	Sex	Race	Locality	Emaciation	Vomiting	Black vomit	Convulsions	Kernig's symptom	Rigidity of neck-muscles	Rigidity of other muscles	Coma	Fever	Duration of illness (hours)	Clinical Character	Termination	Periph. blood	Heart blood	C.-sp. fluid	Urine (alb.)	P.M. hours after death	Jaundice	Pia mater: Hyperaemia	Pia mater: Acute Inflammation	Brain	Spinal-cord	Peripheral Lymph Nodules: Enlarged	Peripheral Lymph Nodules: Hyperaemia	Mesenteric Lymph Nodules: Enlarged	Mesenteric Lymph Nodules: Hyperaemia	Spleen: Enlarged	Spleen: Hyperaemia	Spleen: Haemorrhage	Spleen: Inflammation	Liver: Hyperaemia	Liver: Haemorrhage	Liver: Fat	Liver: Inflammation
1	B.D.	13/I	4	♀	Black	May Penn	−	−	...	+	...	...	+	...	...	3	V.S.	D	...	...	+	...	11	−	+	−	−	...	−	...	+	−	−	−	−	−	+	−	+	+
2	R.B.	13/I	4	♀	Coloured	May Penn	−	+	...	−	−	−	−	+	−	...	V.S.	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3	R.R.	13/I	2	♀	Coloured	May Penn	−	+	...	+	...	...	...	−	+	...	?	R	—	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
4	V.C.	14/I	2	♀	Black	Buff Bay	−	+	...	−	...	...	...	...	−	½	V.S.	D	...	...	+	...	25	−	+	+	+	+	−	...	+	−	−	−	−	−	+	...	...	...
5	A.A.J.	16/I	1 11/12	♂	Black	Porus	+	−	...	−	...	...	...	...	...	2	V.S.	D	...	...	...	...	28	−	+	−	−	−	−	...	−	−	+	−	+	+	−	−	−	−
6	J.R.	18/I	2 2/12	♂	Black	Porus	+	+	...	−	...	...	...	...	+	3 weeks	?	D	...	...	−	...	12	−	+	−	−	−	−	...	−	−	−	−	−	−	−	−	+	−
7	J.J.G.	18/I	8	♂	Black	May Penn	−	...	...	+	...	...	...	+	...	...	V.S.	D	...	...	+	...	26	+	+	−	−	−	−	...	−	...	−	−	−	−	−	−	+	−
8	H.S.	18/I	3 6/12	♀	Black	Chapeltown	+	+	...	−	...	...	+	...	...	2	V.S.	D	...	−	+	...	16	−	+	−	−	−	−	...	+	+	−	−	−	+	−	−	+	...
9	A.S.	21/I	3	♂	Coloured	Kingston	−	+	...	−	−	−	−	−	−	48	V.S.	R	−	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10	S.S.	21/I	4	♀	Coloured	Kingston	−	+	...	−	−	−	−	−	−	48	V.S.	R	−	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
11	A.W.	21/I	8/12	♀	Black	Kingston	...	+	...	−	−	−	−	+	+	48	V.S.	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12	A.M.	21/I	3	♀	Black	St. Andrew	−	+	...	+	−	−	−	+	−	12	V.S.	D	...	−	+	...	16	−	+	−	−	−	−	...	+	+	−	−	−	−	+	−	+	+
13	T.S.	23/I	16	♀	Black	St. Andrew	−	+	...	−	−	−	−	−	−	48	V.S.	R	−	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
14	A.N.	28/I	12	♂	Coloured	St. Mary	+	+	...	−	−	−	−	+	+	72	V.S.	R	−	...	−	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
15	C.N.	28/I	9	♂	Coloured	St. Mary	+	+	−	−	...	...	...	+	−	20	V.S.	D	...	...	−	...	26	−	+	−	−	−	−	...	...	...	+	−	−	+	+	−	+	−
16	C.N.	28/I	5 6/12	♂	Coloured	St. Mary	+	+	−	−	...	−	−	+	−	20	V.S.	D	...	...	−	...	26	−	+	−	−	−	+	...	...	...	+	−	−	+	+	−	+	−
17	M.H.	29/I	27	♀	Coloured	St. Andrew	−	+	...	−	−	−	−	+	+	60	V.S.	D	−	...	−	...	1½	−	+	+	−	−	−	...	−	−	−	−	−	−	−	−	+	...
18	A.B.	29/I	4/12	♀	Black	St. Andrew	...	−	...	+	−	−	−	−	−	?	?	D	...	...	...	...	...	−	+	−	...	...	...	...	...	...	...	...	...	...	...	...	...	...
19	A.H.	18/I	4	♂	Black	Kingston	−	+	...	+	+	+	+	−	−	...	V.S.	D	...	...	+	...	...	...	+	+	...	...	...	...	+	...	...	...	...	...	...	...	+	...
20	T.J.	31/I	12	♂	Coolie	St. Mary	...	...	...	...	...	...	...	...	...	10	V.S.	D	...	−	+	...	12	...	+	−	...	...	...	...	...	...	+	−	−	+	...	+	+	−
21	E.W.	31/I	60	♀	Black	Portland	...	+	...	−	...	...	...	−	−	...	V.S.	R ?	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
22	J.B.	1/II	40	♀	Black	Portland	−	+	...	−	−	+	...	+	−	48	V.S.	D	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
23	C.C.	31/I	20	♂	Chinaman	Portland	−	+	+	...	...	−	−	+	?	5 days	...	D	P.	...	−	+	2½	...	+	−	−	...	...	...	...	...	−	−	−	−	+	−	+	...
24	M.E.	1/II	Adult	♀	Black	Portland	...	+	...	−	−	−	−	−	−	3 days	V.S.	R ?	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
25	R.M.	31/I	10	♂	Black	Portland	...	+	...	...	−	−	−	...	...	24	...	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
26	R.L.D.	1/II	60	♂	Black	Portland	+	+	...	−	...	...	...	...	...	?	...	D	...	−	...	...	...	...	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	...
27	J.M.	1/II	...	...	Black	Portland	...	...	...	...	...	...	...	...	...	...	V.S.	R	...	−	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
28 ?	...	...	...	...	...	Portland	...	...	...	...	...	...	...	...	...	...	V.S.	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
29	B.W.	...	12	♂	Black	Kingston	...	+	...	...	−	−	−	...	...	...	V.S.	D	M.	...	+	...	...	...	+	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...
30	D.B.	31/I	9/12	♀	Black	Kingston	...	+	...	+	+	+	+	+	−	...	•	D	...	...	+	...	...	...	+	−	...	...	...	...	...	...	...	...	...	...	...	...	...	...
31	H.M.	3/II	1 2/12	♂	Black	Kingston	...	+	...	+	+ ?	+	+	+	+	...	•	D	...	...	+	...	...	...	−	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...
32	G.B.	5/II	6	♀	Black	Fourpaths	+	+	...	...	...	...	+	...	...	...	V.S.	D	...	...	...	...	26	...	+	−	−	...	−	...	...	...	−	−	−	−	...	...	...	...
33	E.B.	5/II	2	♂	Black	Fourpaths	+	−	...	−	...	...	...	+	...	...	V.S.	D	...	...	+	...	25	...	+	−	−	...	...	...	+	−	−	−	−	−	−	−	+	−
34	R.S.	6/II	4	♂	Coolie	St. Catherine	−	+	...	+	−	−	−	−	+	...	V.S.	D	...	−	+	+	4	...	+	−	−	...	+	−	...	...	−	−	−	−	−	−	+	+
35	E.M.	6/II	11	♀	Black	Tollgate	−	+	?	+	...	+	+	...	...	...	V.S.	D	...	...	−	...	28	...	+	+	−	...	...	...	+	+	+	+	−	...	−	...	...	...
36	P.B.	6/II	11	♂	Black	Tollgate	+	+	−	−	...	+	...	...	...	...	V.S.	D	...	...	...	...	20	...	+	−	−	...	...	...	+	...	+	+	−	−	−	−	+	...
37	G.B.	7/II	1 4/12	♀	Black	Fourpaths	−	...	...	...	...	+	...	...	...	...	V.S.	D	...	−	•+	...	36	...	+	−	−	...	+	+	+	−	−	−	−	−	+	...	+	...
38	D.L.	8/II	8	♂	...	Kingston	...	+	?	...	...	...	...	...	+	...	V.S.	D	...	...	...	+	...	...	+	−	−	...	...	...	...	...	−	−	−	−	−	−	+	−
39	U.M.	11/II	8	♂	Black	Fourpaths	+	+	...	...	...	...	...	...	...	...	V.S.	D	...	−	+	+	19	...	+	+	−	−	+	−	+	−	−	−	−	−	−	−	+	...
40	A.M.Y.	12/II	2½	♀	Black	St. Andrew	−	−	...	+	−	−	−	+	−	...	V.S.	D	...	•−	+	...	24	...	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+	−
41	M.A.B.	14/II	3	♂	Black	St. Andrew	...	+	...	+	...	...	...	...	...	...	V.S.	D	...	−	−	+	24	...	+	−	−	−	+	+	+	+	−	+	−	−	−	−	+	−
42	C.W.	14/II	2½	♀	Black	St. Andrew	...	+	...	+	...	...	...	+	...	...	V.S.	D	...	...	•+	−	7	...	−	−	−	−	+	+	+	−	+	−	−	+	+	−	+	+
43	C.B.	14/II	2	♀	Black	St. Andrew	...	+	...	+	...	...	...	...	...	...	V.S.	D	...	...	+	...	10	...	+	−	−	−	+	+	+	+	−	−	−	−	−	−	+	...
44	E.P.	15/II	2	♂	Black	St. Andrew	...	+	...	+	...	...	...	...	...	...	V.S.	D	...	−	+	+	...	...	+	−	−	−	+	+	...	...	+	−	−	•	−	−	+	−
45	M.K.	16/II	8	♀	Black	St. Andrew	...	+	...	+	...	+	...	...	...	...	V.S.	D	M.	•	−	...	17	...	+	−	−	−	+	+	+	+	+	+	−	+	−	−	+	−
46	E.B.	17/II	2½	♀	Black	St. Andrew	...	...	...	+	+	+	+	+	...	...	V.S.	D	−	•	•+	...	19	...	+	−	−	−	+	+	+	−	+	−	+	+	−	−	+	−
47	A.W.	18/II	20	♀	Black	St. Andrew	...	+	−	−	−	−	−	+	+	...	V.S.	D	−	−	•+	...	13	...	+	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+
48	L.W.	18/II	12	♂	Black	St. Andrew	...	+	...	...	−	−	−	...	...	...	V.S.	R	−	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
49	M.A.W.	19/II	3	♀	Black	May Penn	...	+	...	+	...	...	...	+	...	...	V.S.	D	...	−	−	...	15	...	+	−	−	−	+	+	...	−	−	−	+	−	+	−	+	−
50	J.M.	21/II	3	♀	Black	St. Andrew	...	...	...	+	−	−	−	+	...	...	V.S.	D	−	−	...	...	17	...	+	−	−	−	+	+	+	−	−	−	+	+	+	−	+	+
51	E.E.E.	23/II	20	♀	Black	St. Andrew	...	...	−	+	−	+	...	+	+	...	V.S.	D	−	−	...	+	19	...	−	−	−	−	+	+	+	+	−	−	−	−	+	−	+	−
52	J.E.	24/II	6	♂	Black	St. Andrew	...	...	...	+	...	...	...	+	...	...	V.S.	D	...	...	−	...	33	...	+	−	−	−	+	+	+	+	−	−	−	•−	+	−	+	...
53	...	23/II	...	♀	Black	St. Andrew	...	+	...	...	...	...	...	...	+	...	?	R	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
54	D.A.	25/II	7	♀	Coloured	St. Andrew	...	+	+	+	...	...	...	+	...	...	V.S.	D	...	−	−	...	19	...	+	−	−	−	+	+	+	−	−	−	−	−	+	−	+	+
55	A.T.	2/III	6	♂	Black	St. Andrew	...	+	...	−	−	+	...	+	...	...	V.S.	D	...	...	+	−	14	...	+	−	−	−	+	+	+	−	−	+	+	+	+	−	+	+
56	R.W.	3/III	...	♀	Black	Kingston	...	...	...	...	...	...	...	...	...	...	V.S.	D	...	−	...	...	...	...	+	−	−	−	+	+	+	+	−	−	−	−	−	−	+	+
57	B.M.	5/III	5	♀	Black	Spanish Town	...	...	...	...	...	...	...	...	...	...	V.S.	D	...	...	+	...	...	...	+	−	+	−	+	+	+	+	−	−	−	+	...	−	+	−
58	D.M.M.	5/III	4	♀	Black	Spanish Town	...	...	...	...	...	...	...	...	...	...	V.S.	D	M.	...	−	−	21	...	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+	−
59	C.W.	6/III	30	♀	Black	St. Andrew	...	+	...	+	...	...	...	+	...	...	V.S.	D	...	...	−	−	8	...	−	−	−	−	+	+	+	−	−	...	−	+	+	−	+	−
60	I.R.	7/III	4½	♀	Black	St. Andrew	...	+	...	+	...	...	...	+	...	...	V.S.	D	...	...	−	+	9	...	+	−	−	−	+	+	+	−	+	+	−	+	+	−	+	−
61	C.W.	8/III	12	♂	Black	St. Andrew	...	−	...	−	...	...	...	...	...	...	V.S.	D	...	...	−	...	31	...	+	−	−	−	+	+	+	−	−	−	−	−	+	−	...	...
62	R.A.L.	9/III	9	♀	Black	St. Andrew	...	...	...	...	...	...	...	...	...	...	V.S.	D	...	...	−	+	...	...	+	+	−	−	+	−	+	−	+	+	−	+	−	−	+	−
Column No.		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)	(28)	(29)	(30)	(31)	(32)	(33)	(34)	(35)	(36)	(37)	(38)	[illegible]

V.S. = Vomiting Sickness D = Death R = Recovery M. = Malaria P. = P[illegible]

BSERVED IN JAMAICA, PRINCIPALLY VOMITING SICKNESS

[illegible]	[illegible]	Mesenteric Lymph Nodules		Spleen			Liver					Pancreas					Kidneys							Stomach			Duodenum			Intestines									Lungs			
[illegible]	[illegible]	Hyperaemia	Enlarged	Hyperaemia	Haemorrhage	Inflammation	Hyperaemia	Haemorrhage	Fat	Inflammation	Necrosis	Hyperaemia	Haemorrhage	Fat	Necrosis	Inflammation	Hyperaemia	Haemorrhage	Fat	Necrosis	Inflammation	Adrenals	Tonsils	Hyperaemia	Petechiae	Black content	Hyperaemia	Petechiae	Inflammation	Hyperaemia	Petechiae	Inflammation	Ascaris	Trichocephalus	Ankylostoma	Pericard petechiae	Myocard fatty change	Hyperaemia	Pechymoses	Infarcts	Oedema
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M. = Malaria

P. = Paraplasma flavigenum

* = vide Text

§ Tuberculosis of lungs and other organs

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EXPLANATION OF PLATES

PLATE XXIX

Figs. 1-2. Two huts in which cases of vomiting sickness occurred. These figures show the very primitive structure and poor appearance of many of the native huts and their situation in the bush.

Plate XXX

All figures are from safranin-stained sections of specimens fixed in Fleming's strong osmic acid solution, and all are drawn by means of Abbe's drawing apparatus, the optic system being Zeiss's apoch. 4 mm. and compensating ocular 4. × 360. All figures on this and the following plate have been drawn by Mrs. Margrethe Seidelin.

Fig. 1. Stomach. Marked fatty change of epithelial cells.

Fig. 2. Pancreas. Marked fatty metamorphosis; small and medium-sized fat droplets are present in practically all the epithelial cells of the alveoli. A Langerhan's islet is shown in the figure, but it is not very sharply defined; its cells have undergone fatty change, but less marked than in the case of the alveoli. The epithelia of the excretory ducts are in particular severely affected.

Fig. 3. Myocardium. Moderate fatty metamorphosis.

Fig. 4. Liver. Intense fatty metamorphosis.

Plate XXXI

Technique as indicated for Plate XXX (× 360).

Fig. 1. Suprarenal capsule (Case 55) cortex. A considerable amount of fat is present in nearly all epithelial cells.

Fig. 2. Kidney (Case 62). Moderate fatty change, both convoluted tubules and glomeruli cells being affected, the latter less than the former, but in the case of both only minute fat droplets are observed in the cells.

Fig. 3. Kidney (Case 47). Intense fatty metamorphosis, the epithelia of the convoluted tubules contains very large fat drops; in the lining epithelia of Bowman's capsules and the glomeruli cells fatty change is also observed, but much less marked.

Fig. 4. Kidney (Case 47). Intense fatty metamorphosis of the epithelia of the ascending limbs of Henle's loops, whilst in the descending limbs the change is very little marked, only some of the epithelial cells showing minute fat droplets.

Plate XXXII

Microphotographs

Fig. 1. Mucosa of stomach (Case 55), showing hyperaemia, diffuse lymphoid infiltration, and superficial necrosis and haemorrhages. To the right, part of a lymphoid follicle is seen. × 95.

Fig. 2. Mucosa of duodenum (Case 34), showing hyperplasia of Brunner's glands and diffuse microcellular infiltration; also superficial necrosis. Remnants of the muscularis mucosae are seen, but this coat has been entirely broken up by the proliferated glands. × 95.

Fig. 3. Liver (Case 55), showing intense diffuse fatty metamorphosis and moderate necrobiotic changes, likewise of a diffuse character (× 370). Other parts of the same liver showed intense capillary hyperaemia.

Fig. 4. Pancreas (Case 43), showing marked necrobiotic changes. × 370.

Plate XXXIII

Microphotographs

Fig. 1. Hyperaemia of lymph nodule. × 140.

Fig. 2. Spleen: Hyperaemia. × 140.

Fig. 3. Kidney: Necrobiotic changes. × 370.

Fig. 4. Kidney: Intense fatty change. × 370.

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The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1914:—

Full Course begins 6 January. Advanced Course begins 1 June.
Diploma Examination, 6 April. Certificate Examination, 30 June.
Full Course begins 15 September.
Diploma Examination, 14 December.
These dates are subject to revision.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

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EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the fourth number of the seventh volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

STUDIES IN BLACKWATER FEVER

(1) STATISTICAL

BY

J. W. W. STEPHENS, M.D. (CANTAB.)

In this paper I propose to consider some of the facts bearing on the malarial origin of blackwater fever which I have been able to collect from an examination of recent literature.

Arguments of a general nature, though there are many, I shall not consider here, but shall examine the facts so far as I have been able to ascertain them under the following headings:—

(1) Malaria parasites.
(2) Pigmented leucocytes.
(3) Post-mortem examinations.
(4) Influence of malaria.
(5) Relationship to species of malaria parasite.
(6) Effect of period of residence.
(7) Seasonal prevalence.
(8) Correlation between malaria and blackwater statistics.
(9) Second attacks.

In 1901, Christophers and myself (1901), as a result of our microscopical studies of blackwater fever, came definitely to the conclusion that blackwater is malarial in origin but is not simply an attack of malaria, but occurs only in those who are in a condition induced by repeated malarial infection lasting over a certain time.

MALARIA PARASITES

One of the most important facts, in my opinion, bearing on the aetiology of blackwater fever is the presence of malaria parasites in the blood of blackwater cases. In the literature I have been able to collect 390 cases. I divide them into three categories, those on the day before, those on the day of, and those on the day after the onset of blackwater, as in the accompanying Table I. My reason for doing this is that in my experience, and also in that of others, parasites when found rapidly disappear.

TABLE I

Author	Day before onset of hemoglobinuria		Day of onset of hemoglobinuria		Day after onset of hemoglobinuria		Remarks
	No. of cases	No. positive	No. of cases	No. positive	No. of cases	No. positive	
Barratt & Yorke (1909)	2	1	5	0	7	0	
Brault (1903)	—	—	1	1	—	—	It is not clear whether 'day of' or 'day after.'
Brem (1906)	1	1	1	0	1	0	Cases 10 and 13; for other cases see Deeks & James.
Capogrossi (1910)	—	—	1	1	1	0	S.T. parasites present on 1, 4, 7, and 8th day.
Cardamatis (1911)	—	—	15	0	4	2	
Carducci (1907)	—	—	1	1	1	1	Bl. pos. on 1, 2nd day, neg. on 3, 4, 5th day, death.
Christophers & Bentley (1908)	—	—	10	6		—	
da Costa (1906)	2	2	12	10	19	10	
Deaderick (1907)	—	—	4	1	2	0	
Deeks & James (1911)	28	14	36	12	42	7	
Frere (1910)	—	—	1	1	1	0	
Gastou & Dufougeré (1911)	—	—	1	1	—	—	Not clear whether 'day of' or 'day after.' 10 c.cm. blood centrifugalised and crescents found.
German East Africa (1909-10)	1	1	2	2	2	1	
Grattan (1907)	—	—	1	0	2	1	
Jungels (1911)	—	—	1	0	1	0	
Kleine (1901)	2	2	2	1	1	0	

TABLE I—*continued*

Author	Day before onset of hemoglobinuria		Day of onset of hemoglobinuria		Day after onset of hemoglobinuria		Remarks	
	No. of cases	No. positive	No. of cases	No. positive	No. of cases	No. positive		
Krauss (1904)	—	—	—	—	1	0		
Külz (1908)	—	—	—	—	1	0		
Külz (1910)	—	—	—	—	1	1		
Marshall (1910)	—	—	—	—	1	0		
Masterman (1906)	—	—	—	—	1	1		
da Matta (1912)	2	1	1	0	—	—		
O'Donoghue (1912)	1	1	1	0	1	0		
Orme (1908)	—	—	—	—	1	1		
Otto (1902)	1	1	1	0	1	0		
Powell (1898)	2	2	—	—	—	—		
Römer (1911)	—	—	—	—	1	0		
Ross, Thomson & Simpson (1910)	1	0	1	0	1	0		
Ruge (1902)	1	1	1	1	1	0		
Stephens (1903)	23	22	63	39	64	11		
Whipple (1909)	—	—	—	—	2	1		
							Total cases	Positive
	67	49	162	77	161	37	390	163
	73%		47·5%		23%		41·8%	

This means that in 100 cases of blackwater examined on the day before the onset, parasites will be found in 73 cases, in 100 cases examined on the day of onset in 47·5* and in 100 cases examined on the day after in 23.†

An important deduction that I think may safely be made from these figures is that parasites disappear, and indeed rapidly, during the course of the disease, as we had observed in our original studies. This disappearance of parasites is in all probability associated with the acute haemolysis that is taking place in blackwater fever, and *should be borne in mind in all comparisons* between the parasite rates in blackwater fever and malaria.

Further, the fact that on the day before the onset of the blackwater parasites are found in 73% of cases seems to point to the initial attack being closely associated with a malarial attack. That relapses do occur during the course of the disease (which relapses in some cases at least can be brought on by quinine) without the occurrence of parasites in the blood, seems to be certain, though I have not collected the data on the subject. And even if parasites were rarely found in blackwater, it would not necessarily exclude its malarial origin, because it might be that it was just in the chronic cases of malaria where parasites are absent or scanty that blackwater occurred, but I will not pursue the argument further, as I wish as far as possible in this paper to ascertain facts and avoid discussion.

The following table, compiled from Panse's (1902) cases, 35 in number, enables one to show in more detail how time affects the result of the examination for parasites in blackwater:—

TABLE IA

Time of blood examination	Cases	Positive	Percentage
Day before onset	9	8	88·9
Day of onset (*a*) before onset	11	8	72·7
,, (*b*) after onset	11	6	54·5
Within 12 hours after onset	12	6	50·0
More than 12 hours after onset	31	9	29·0

* A correction is probably necessary for those cases occurring on the day *of* the blackwater for some of these will probably have been examined *before* the onset of the blackwater and some (probably the majority) after, so that on the assumption that the parasite rate before the onset and that of the day before is the same, this figure should be reduced, but it is impossible to say by how much.

† Or put in a different way of 100 *positive* cases, 51 will occur on the day before, 32·8 on the day of, and 16 on the day after the blackwater.

The next tables, Table II and III, I give separately, as the data were compiled in a different way to that in Table I.

Table II.—Parasites in Blackwater Fever. Compiled from Deeks and James (1911)

75	cases	examined	4 — 1 days *before* onset	...	Pos.	48 = 64·0%
28	,,	,,	1 day *before* ,,	...	,,	14 = 50·0%
36	,,	,,	*day* of *onset* ...	...	,,	12 = 33·3%
62	,,	,,	1 — 4 days *after onset*	...	,,	12 = 19·4%
40,928	,,	of	Malaria examined on admission	...	,,	23,410 = 57·2%
260	,,		Amoebic dysentery		,,	48 = 18·5%
70	,,		Liver abscess		,,	7 = 10·0%
33	,,		Typhoid		,,	4 = 12·1%
— ?	,,		Tuberculosis			About 12·0%
— ?	,,		Pneumonia			Less than 12·0%

Table III

Madeira-Mamore Railway Co., Porto Velho, Brazil. Compiled from Lovelace (1913)

	Total blood-examinations (26 months)	Positive	Rate
Malaria	16,434	9,155	55·7%
Blackwater*	383	178	46·5%

The tables are also of value because we are able to compare the percentages in blackwater with those made in malaria under the same conditions of observer, staining, and probably of time devoted to each examination.

In the next table, Table IIIA, the data of the first three tables are summarized.

From it we see that parasites are found in blackwater fever in 42·9% of cases (taking no account of the day of the disease on which the examination is made), while in malaria, parasites are found in 56·8% of cases. If we accept the conclusion drawn from Table I, viz., that parasites disappear during the course of the

* In the majority of cases only one blood examination was made, and it was made during the period of haemoglobinuria.

disease, the lower percentage in blackwater is readily accounted for. The figure for malaria is probably low, but in the Panama figures of Deeks and James it should be remembered that only a single examination was made on admission to hospital.

Table IIIa.—Parasite Rate in Blackwater Fever and Malaria

Cases	Positive	%	Observer
Blackwater Fever			
390	163	41·8	Various observers (Table I)
230	89	38·7	Deeks & James (1911)
383	178	46·5	Lovelace (1913)
1003	430	42·9*	
Malaria			
40,928	23,410	57·2	Deeks & James (1911)
16,434	9,155	55·7	Lovelace (1913)
57,362	32,565	56·8	

The objection has been brought against figures of this kind that in a malarial country parasites would be found in the same or about the same percentage in people not suffering from blackwater fever, but such objections have *not* been supported by actual data.

Christophers and myself (1901), moreover, examined 44 Europeans at Lagos, taken at random. They were especially from communities suffering much from malaria. We found parasites in 2, pigmented leucocytes in 2, i.e, 4 infected out of 44, about 9%.

Christophers and Bentley (1908) examined 66 babus in the Duars, Bengal, and found parasites or pigment in 14, i.e., 21·2%. In 10 cases of blackwater on the day of onset they found parasites or pigment in 8, or 80%. Further we have the control figures of Table II.

* This figure represents the positive parasite rate irrespective of the day of the disease and should be compared with the figures of Tables I and II.

If, then, these data are correct, they imply that in the general population and those suffering from diseases other than malaria or blackwater fever the parasite rate is not comparable with that in blackwater fever.

Moreover, I believe we are justified in meeting the above objection more forcibly still. We have in blackwater a disease in which immediately before the onset of the disease malaria parasites are found in 73% of cases, taking the figure of Table I. If this does not signify that the disease is malarial, then the finding of parasites in 73% of cases of 'malaria' has also no significance, and 'malaria' is also due to some unknown cause.

PIGMENTED LEUCOCYTES

In blackwater fever it is the general experience that parasites when found rapidly disappear. We still, however, have a means of detecting a malarial infection if present, viz., by means of pigmented leucocytes. In the literature most frequently no statement is made with regard to pigmented leucocytes. The following figures of Christophers and Bentley are small and only suggestive, and more observations are required.

Table IV.*—Pigmented Leucocytes in Blackwater Fever (Christophers & Bentley, 1908).

	First Day		Second Day		Fourth-Sixth Day	
	No. of cases	No. positive	No. of cases	No. positive	No. of cases	No. positive
Parasites	10	6	7	0	10	1
Pigment	10	8	7	6	10	4

POST-MORTEM EXAMINATIONS

In 31 cases where definite statements positive or negative have been made malaria pigment was found P.M. in 26, i.e., 83·9%. The evidence of malaria then in 5 cases is negative. If the absence of pigment P.M., supposing the facts correct, definitely excludes malaria then some blackwater cases must be due to other causes, which one must admit is not impossible, although I believe the facts prove that malaria is the dominant factor.

* In this table, the data are obtained from the same 10 cases.

TABLE V.—Post-mortem Records of those cases only where definite statement made.

Cases	Parasites or pigment positive	Negative	Authority
3	0	3	Barratt & Yorke (1909). But in one of these scanty pigmented leucocytes, found during life.
6	6	0	H. Werner (1907).
13	13	0	G. H. Whipple (1909).
2	2	0	Christophers & Bentley (1908).
5	5	0	Stephens & Christophers (1901). (B.W. cases IX-XVI, p. 24.)
2	0	2	Brem (1906). In one case only a smear from a rib examined.
31	26	5	
	83·9%	16·1%	

INFLUENCE OF MALARIA

The influence of malaria in determining blackwater is shown in a different way by the following table compiled from Deeks and James.

TABLE VI.—Influence of Malaria. Ancon Hospital, July, 1904—Sept., 1910.

Among 40,928	cases diagnosed as Malaria,
102	cases of Blackwater developed subsequent to admission.
Among 42,000	cases diagnosed as Typhoid, Pneumonia, Amoebic dysentery, and Tuberculosis, etc. (medical and surgical).
4	cases of Blackwater developed subsequent to admission.*

RELATIONSHIP TO SPECIES OF MALARIA PARASITE

The statistics on this point are often fallacious. What we require to know is not the percentage of malignant tertian or other species of parasite in blackwater cases, but rather what number of blackwater cases there is in 100 cases of malignant tertian, simple tertian and quartan respectively. The following figures show this.

* In the surgical wards: 3 after well defined malarial paroxysms, 1 after quinine in a patient with a history of much malaria.

TABLE VII.—Compiled from Deeks & James (1911)

	Of 23,410 positive malaria cases	Of 89 positive blackwater cases	Ratio of blackwater % to malaria %	Ratio of M.T. to S.T.
Malignant Tertian	74 %	76·4 %	103·2 %	100
Simple Tertian	26 %	23·6 %	90·8 %	88

i.e., if x cases of malignant tertian malaria give 100 cases of blackwater.
then x ,, simple ,, ,, ,, 88 ,, ,,

Compiled from Lovelace (1913)

	Of 9,155 positive malaria cases	Of 178 positive blackwater cases	Ratio of blackwater % to malaria %	Ratio of M.T. to S.T. and to M.T. & S.T. respectively
Malignant tertian	5988 = 65·4 %	87 = 48·9 %	74·8 %	100
Simple tertian	2760 = 30·1 %	74 = 41·6 %	138·2 %	185
M.T. + S.T.	391 = 4·3 %	17 = 9·6 %	223·3 %	299
Quartan	16 = 0·2 %	0 = 0·0	0·0	0

i.e., if x cases of malignant tertian malaria give 100 cases of blackwater.
then x ,, simple ,, ,, ,, 185 ,, ,,
and x ,, M.T. + S.T. ,, ,, 299 ,, ,,
and x ,, quartan ,, ,, 0 ,, ,,

The discrepancy between these two results I cannot at present explain, assuming they are both correct, but it should be noted that Lovelace (1913) in his paper states that the simple tertian parasite 'is here an extremely persistent infection. More time and more quinine are required for its eradication than for that of the aestivo-autumnal parasite.' And again he speaks of 'the rapid blood destruction in, the great prostration incident to, and the diabolical persistence of tertian infections.' It would seem as if here we had an example of increased virulence of the simple tertian parasite. One's own clinical experience of the malignant tertian parasite in the tropics and in temperate zones, seems certainly to point to differences in virulence.

INFLUENCE OF PERIOD OF RESIDENCE

There is a general impression that blackwater is most prevalent in the second or third year of residence, but the statistics on this point are nearly all subject to the objection that no account has been taken of the number of people in each residential period, first, second, third, etc., years, and this may vary much from year to year. Here again, what we require to know in the first place is not out of one hundred cases of blackwater how many occur in the first, second, etc., years, but how many cases of blackwater occur in each one hundred people or similar sample of the different periods of residence, one, two, three, etc., years. Knowing this, we can then calculate how many out of one hundred cases of blackwater occur in the first, second, third, etc., years. Plehn (1901) is the only observer who supplies us with what we require to know, though, unfortunately, his figures extend only to two years.

The figures in the second row of Table VIII indicate the number of people in their first, second, third, etc., month of residence under observation (for Hgb. estimations). Thus in the second month of residence there were seventy-five, therefore eight of these were not under observation in their first month, but whether the remaining sixty-seven were the same people as the sixty-seven in their first month, the table does not tell us; probably they were, and at the end of twenty-four months they were reduced to five, but it is not a material point.

TABLE VIII.—Blackwater Fever: Effect of Residence (Plehn, 1901).

FIRST YEAR

Month...	1	2	3	4	5	6	7	8	9	10	11	12
Residents	67	75	75	71	67	62	61	57	55	50	46	47
Malaria cases	28	50	60	42	41	43	38	44	37	33	26	24
B.W. cases	0	0	0	0	0	3	4	6	3	4	1	1

Second Year

Month...	13	14	15	16	17	18	19	20	21	22	23	24
Residents	42	42	37	35	33	25	21	17	12	10	9	5
Malaria cases	21	29	18	9	13	12	7	9	1	3	1	4
B.W. cases	1	4	2	0	1	1	0	1	0	0	0	2

Table VIII. B. (compiled from previous table.)

Time of Residence	First 6 months	First year	Second year
Number of residents	69·5	61·1	24
Cases of blackwater	3	22	12
B.W. rate per 100 residents	4·32	36·0	50·0
Of 100 cases of B.W. there occur	5·02	41·86	58·14

i.e., Among a population of 100 4·32 cases will occur in 1st 6 months residents.
36·0 ,, ,, 1st year ,,
50·0 ,, ,, 2nd year ,,
And of 100 cases of B.W. occurring during the 2 years' period :
5·02 will occur in the 1st 6 months.
41·86 ,, ,, 1st year.
58·14 ,, ,, 2nd year.

Malaria cases	264	466	127
B.W. cases	3	22	12
B.W. rate per 100 malaria cases	1·14	4·72	9·45

i.e., For 100 cases of malaria in 1st 6 months' residents there will be 1·14 blackwater.
,, ,, ,, 1st year's ,, ,, 4·72 ,,
,, ,, ,, 2nd ,, ,, ,, 9·45 ,,
And of 100 cases of B.W. in 2 years there will be 8·05 among x malarial cases in the 1st 6 months.
33·31 ,, ,, ,, 1st year.
66·69 ,, ,, ,, 2nd year.

The next table gives the distribution of 1,050 cases of which I have found records. The data for the first six months and first year, and possibly second year, are probably fairly correct, as they have been taken from large and probably approximately equal populations. But in the third and subsequent years we do not know what proportion the population bears to that of the first year, and as I have already pointed out, this is essential before a comparison can be made. I should note, however, that Daniels in his figures has made this correction, and that the difference from the uncorrected figures was only slight.

TABLE IX.—Blackwater Fever: Effect of Residence.

First 6 months	First year	Second year	Third year	Fourth year	Fifth year	Later	Reference
1	1	4	—	1	1	3	Barratt & Yorke (1909).
?	10 (5·4 %)	42 (22·5 %)	79 (42 %)	37 (20 %)	9 (4·8 %)	?	Bérenger Féraud (1874).
2	3	2	—	1	—	—	Brem (1911).
1	2	5	6	1	1	3	Broden (1906).
?	6	22	43	20	9*	—	Burot & Legrand (1897). * Longer than 4 years.
—	—	7	1	3	—	3	Campenhout & Dryepondt (1901).
7	7	23	?	?	?	?	Cardamatis (1902).
—	8	40	12	5	1	—	Christophers & Bentley (1908).
—	1	3	3	2	5	2	da Costa (1906).
4	21	40	27	12	5	9	Daniels (1901).
39	71	79	38	12	5	4	Deeks & James (1911).
—	—	—	—	—	—	1*	Howard (1907). * First attack after 24 years.
2	7	2	1	—	2	3	Koch (1899).

TABLE IX.—Blackwater Fever: Effect of Residence—*continued.*

First 6 months	First year	Second year	Third year	Fourth year	Fifth year	Later	Reference
?	2	3	2*	2*	2*	39	McElroy (1905). *Six cases during 3rd, 4th, and 5th years.
—	—	13*	—	—	—	—	Mense (1899). *All had been in Africa at least 18 months.
4	10	3	3	—	—	3	Panse (1902).
12	42	41	14*	—	—	—	Plehn, A. (1903). *Later than the 2nd year.
3	9	8	4	1	—	—	Plehn, F. (1898).
?	17	?	?	?	?	?	Poole (1899).
1	2	2	—	—	—	1	Reynalds (1899).
?	5	7	28	5	2	4	Védy, cited by McElroy (1905).
76	224	346	261	102	42	75 = 1,126 Less 76 during first 6 months. 1,050 cases.	
7·2%	21·3%	33·0%	24·9%	9·7%	4%	7·1%	

I believe that we may deduce from these figures the fact that blackwater is not a disease of the first six months or first year, but mainly of the second year. I believe this fact is capable of a simple explanation, viz., that blackwater fever only shows itself in the majority of cases when the patient has been subjected to 'repeated malarial infection lasting over a certain time.'

THE SEASONAL PREVALENCE

In Tables XA, XB the distribution of 226 European cases of blackwater fever in Northern Nigeria (1912) month by month is given. The figures have not been corrected for monthly variations in the European population as no figures are available for this purpose. From 1900-1911 the total European population has increased from about 175 to 775, but there is no reason to suppose that the increase has not been a uniform one, spread over the whole of each year, or that if in any year any disproportionate increase occurred in any particular month it would not be equalized in other months in other years.

TABLE XA.—N. Nigeria. Showing the distribution by month of blackwater cases from 1899-1911.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1899	10	2	1	...	...	...	...	2	2	...	1	2	20
1900	2	2	3	...	...	1	1	1	...	...	1	1	12
1901	1	...	...	...	2	1	1	2	3	...	2	...	12
1902	4	...	1	1	...	2	1	3	1	3	2	2	20
1903	...	...	1	1	...	...	2	2	3	3	2	3	17
1904	3	3	1	...	4	2	...	4	6	5	1	6	35
1905	1	...	2	...	1	1	1	3	1	3	3	4	20
1906	3	1	...	2	...	...	5	2	4	3	2	4	26
1907	...	3	2	...	1	...	1	3	...	...	...	2	12
1908	1	...	1	1	...	...	2	1	3	2	3	2	16
1909	1	2	1	2	...	1	1	2	...	1	2	1	14
1910	2	1	...	...	...	...	1	...	2	2	1	1	10
1911	2	...	1	2	...	1	2	2	1	1	...	...	12
Total	30	14	14	9	8	9	18	27	26	23	20	28	226

TABLE XB.—Northern Nigeria

Months	Actuals	Corrected for a month of 30·4368 days	Departure + or − from average 18·8
1	30	29·5	+ 10·7
2	14	15·2	− 3·6
3	14	13·7	− 5·1
4	9	9·1	− 9·7
5	8	7·9	− 10·9
6	9	9·1	− 9·7
7	18	17·7	− 1·1
8	27	26·5	+ 7·7
9	26	26·4	+ 7·6
10	23	22·6	+ 3·8
11	20	20·3	+ 1·5
12	28	27·5	+ 8·7
	226	225·5	

Column 1 represents the months. Column 2 the actuals, taken from the previous table. Column 3 the corrected figures for a month of 30·4368 days. Column 4 the departure + or — from the average figure 18·8. We observe in this table that the cases *below* the average come together, and Mr. Stott has calculated for me that it is 131 to 1 against the distribution being simply a random one. Further, he has calculated that if we take into account the *amount* of departure, that it is 1,000 to 1 in favour of the effect being due to season.

The next Tables XIA, XIB show similar figures for Southern Nigeria, Graham (1912).

TABLE XIA.—Southern Nigeria. Showing the distribution by month of blackwater cases from 1899-1911.

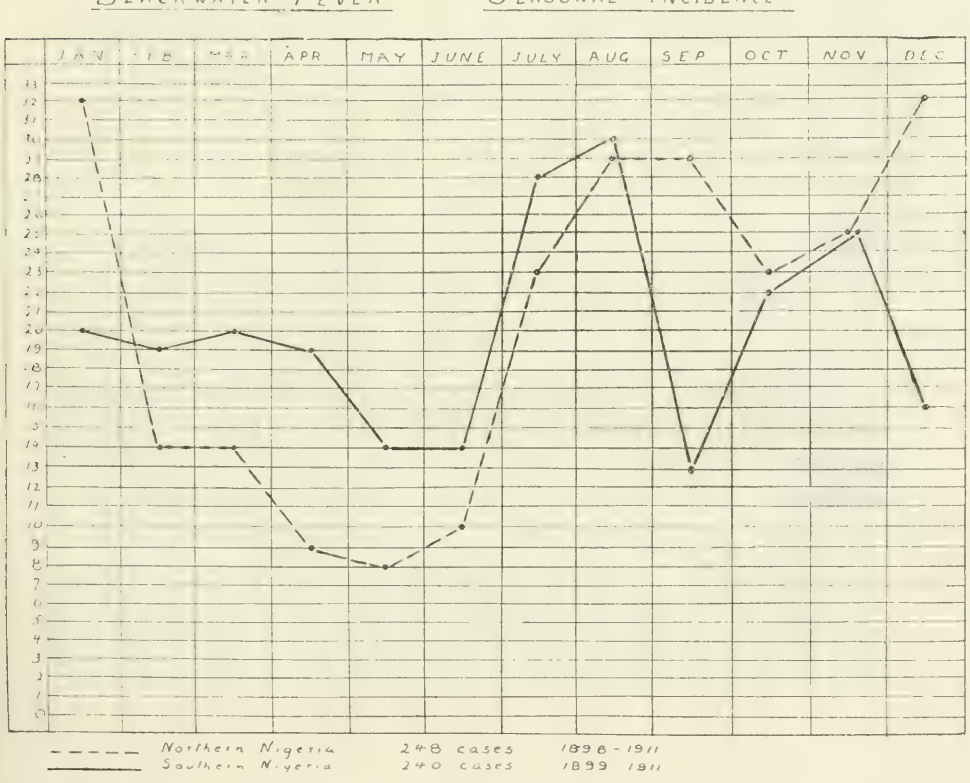
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1899	...	...	...	...	1	...	1	2	...	...	...	1	5
1900	...	...	...	1	1	...	1	...	...	...	...	...	3
1901	2	2	...	1	1	1	2	5	1	...	3	2	20
1902	5	3	3	1	1	...	1	...	1	5	3	...	23
1903	1	1	2	2	1	2	1	3	1	1	2	1	18
1904	...	2	...	2	2	2	4	3	...	1	...	1	17
1905	1	1	2	1	2	3	...	2	...	1	...	1	14
1906	1	3	1	2	1	2	2	3	3	3	4	...	25
1907	5	...	1	1	1	1	5	3	3	1	4	2	27
1908	3	2	4	4	1	1	3	1	1	1	...	...	21
1909	1	2	2	1	...	2	1	3	2	...	...	2	16
1910	1	1	3	2	1	...	3	3	...	5	6	5	30
1911	...	3	2	1	1	...	4	2	1	4	3	1	22
Total	20	20	20	19	14	14	28	30	13	22	25	16	241

TABLE XIB.—Southern Nigeria.

Months	Actuals	Corrected figures for a month of 30·4368 days	Departure + or − from the average 20·1
1	20	19·6	− ·5
2	20	21·7	+ 1·6
3	20	19·6	− ·5
4	19	19·3	− ·8
5	14	13·7	− 6·4
6	14	14·2	− 5·9
7	28	27·4	+ 7·3
8	30	29·5	+ 9·4
9	13	13·2	− 6·9
10	22	21·6	+ 1·5
11	25	25·4	+ 5·3
12	16	15·7	− 4·4
	241	240·9	

Column 1 represents the months. Column 2 the actuals, taken from the previous table corrected for a month of 30·4368 days. Column 3 the departure + or — from the average figure 20·1. In this case Mr. Stott remarks ' some other factor seems to be at work and the probability is reduced to 7 to 1.'

CHART A



The next table represents similar figures for the European cases of blackwater admitted into Ancon Hospital.

TABLE XII.—Ancon Hospital, Panama. Showing the distribution by month of blackwater cases among Europeans from 1908-1912.

	1908	1909	1910	1911	1912	Totals	Departure + or − from the average 20
January	3	17	1	5	2	28	+ 8
February ...	1	9	4	6	3	23	+ 3
March	3	10	3	4	3	23	+ 3
April	1	6	3	8	1	19	− 1
May	0	7	3	4	1	15	− 5
June	0	3	4	7	1	15	− 5
July	1	0	3	4	5	13	− 7
August	1	4	3	8	4	20	0
September ...	0	1	2	10	6	19	− 1
October	8	3	4	5	3	23	+ 3
November ...	11	7	3	6	2	29	+ 9
December ...	5	1	7	0	1	14	− 6
						241	

In Table XIII, I have given the uncorrected totals of Table XII and the totals corrected for monthly variations in the European population to show that the general conclusion is not materially affected.

TABLE XIII.—Europeans at Ancon Hospital.

	Average European Population, 1908-1912	Uncorrected		Corrected for population and for length of months		
		Blackwater cases admitted 1908-1912	Deviations from average	Blackwater rate per 10,000	Calculated to make 241	Deviations from average
1	6,061	28	+ 8	46·18	27·8	+ 7·7
2	6,112	23	+ 3	37·63	25·1	+ 5·0
3	6,090	23	+ 3	37·77	22·8	+ 2·7
4	6,436	19	− 1	29·52	18·4	− 1·7
5	6,324	15	− 5	23·72	14·3	− 5·8
6	6,143	15	− 5	24·42	15·2	− 4·9
7	6,138	13	− 7	21·18	12·8	− 7·3
8	5,982	20	0	33·43	20·1	− 0·0
9	5,953	19	− 1	31·92	19·8	− 0·3
10	6,241	23	+ 3	36·85	22·2	+ 2·1
11	6,071	29	+ 9	47·77	29·7	+ 9·6
12	6,290	14	− 6	22·26	13·4	− 6·7
	73,841	241		392·65	241·6	
Average ...	6,153	20			20·1	

Accordingly, in Panama also, as Mr. Stott observes, we have six cases below the average coming together, making a probability of 131 to 1 in favour of a seasonal incidence, although in this case our confidence is reduced by one-third owing to the average being only over 5 years, instead of 13.

A similar conclusion can also be drawn from the figures for blackwater for all employees admitted to the Commission hospitals (Table XIV) showing that the Ancon figures are a fair sample of the conditions existing over the whole zone.

For comparative purposes the monthly distribution of malaria is also given, in which the seasonal malarial rise is clearly shown. It should be noted that there are slight discrepancies between the returns (Table XII) from Ancon Hospital (Europeans), privately communicated by Dr. James, and those given in the monthly official reports. I believe that the Ancon figures are reliable, as they are the hospital records, whereas it is easily intelligible that errors may occur in the official figures for the whole zone.

TABLE XIV.—Showing the monthly distribution of malaria and blackwater among *all* employees admitted to the Isthmian Canal Commission Hospitals, 1907-1912.

MALARIA

	1907	1908	1909	1910	1911	1912	Total	Departure from average (5,226)
January	1,822	641	1,245	479	331	496	5,014	− 212
February ...	1,607	570	832	354	375	502	4,240	− 986
March	1,570	525	657	528	402	343	4,025	−1,201
April	822	402	524	436	319	235	2,738	−2,488
May	607	393	576	613	920	257	3,366	−1,860
June	928	629	737	1,261	1,736	424	5,715	+ 489
July	1,669	1,721	944	1,750	1,971	1,035	9,090	+3,864
August	2,179	1,518	907	1,417	896	915	7,832	+2,606
September ...	1,809	1,404	963	883	651	438	6,148	+ 922
October	1,594	1,815	1,071	614	496	301	5,891	+ 665
November ...	1,019	1,447	852	558	404	272	4,552	− 674
December ...	803	1,225	763	553	385	374	4,103	−1,123
Total ...	16,429	12,290	10,071	9,446	8,886	5,592	62,714	

BLACKWATER FEVER

								(Average, 33·7)
January	3	5	13	1	8	4	34	+ 0·3
February ...	8	4	20	4	9	8	53	+ 19·3
March	6	8	9	4	5	3	35	+ 1·3
April	4	5	11	2	10	2	34	+ 0·3
May	2	4	9	7	8	2	32	− 1·7
June	2	1	6	2	9	4	24	− 9·7
July	3	4	2	7	10	2	28	− 5·7
August	2	7	2	7	11	4	33	− 0·7
September ...	2	6	7	3	13	...	31	− 2·7
October	2	7	3	5	7	...	24	− 9·7
November ...	3	13	7	3	10	...	36	+ 2·3
December ...	1	19	9	8	1	2	40	+ 6·3
Total ...	38	83	98	53	101	31	404	

TABLE XV.—Malaria among the European Troops in German East Africa.

	1893-94	1894-95	1895-96	1896-97	1897-98	1898-99	1899-1900	1900-01	1901-02	1902-03	1903-04	1904-05	1905-06	1906-07
Strength	—	123	111	104	112	105	129	130	150	162	130	138	242	189
Malaria: Cases ...	—	412	414	343	318	345	386	261	229	209	162	140	252	158
	316%	335%	373%	330%	284%	329%	299%	201%	153%	129%	125%	101%	104%	84%
Deaths ...	—	1	—	2	0	1	0	1	1	1	0	0	0	0
Blackwater Fever:														
Cases ...	—	4	32	19	30	32	19	17	12	8	9	2	6	5
Deaths ...	—	?	7	2	2	2	11	3	1	0	1	1	0	0

Most of the statistics of blackwater and malaria do not extend over a sufficiently long period to enable one to say that the correlation between the two is sufficiently close to make it fairly certain that these are connected, or both subject to the same influence. This applies, for example, to the figures for German East Africa, Schilling (1910). An examination of these figures, kindly made for me by Mr. Stott, Hon. Statistician to the School, certainly suggests a correlation, but the period of years over which they extend is too small to give a sufficiently satisfactory probability.

The next table—Table XVI—shows the amount of malaria and blackwater in Panama in blacks and whites from 1906 to 1912. All the table shows is that there has been a steady fall in malaria, and also a fall in blackwater, but one cannot argue that there is necessarily any relationship between the two, as the period is too short. Ten years hence these figures with the additional observations should be valuable. A probable fallacy is that we are not dealing with a similarly constituted population each year, owing to large emigration and immigration of labourers. If, for instance, period of residence is a factor determining blackwater, then if the number of people of each period of one, two, three, etc., years varies each year, the blackwater rate may vary quite independently of the malaria rate.

Table XVI.—Malaria and Blackwater Fever in the Panama Canal Zone.

	Employees' Annual Average			Malarial Admission Rate per 1,000 per annum			Blackwater Admission Rate per 1,000 per annum		
	Total	White	Black	Total	White	Black	Total	White	Black
1906	26,547	5,400	21,147	821·0	940·7	787·7	1·88 ?	—	—
1907	39,238	10,709	28,429	418·7	753·6	293·9	0·97	—	—
1908	43,890	12,383	31,507	280·0	507·8	192·4	1·89	5·09	0·63
1909	47,167	11,662	35,505	211·4	366·2	163·3	2·08	6·52	0·62
1910	50,802	13,021	37,781	185·9	372·7	121·5	1·04	3·07	0·34
1911	48,876	12,251	36,625	181·8	334·1	130·8	2·07	6·61	0·55
1912	50,893	12,553	38,340	109·8	216·7	74·8	0·61	1·99	0·16

But a serious drawback to this table is that the statistics regarding Americans and Europeans are massed together. The malaria and blackwater fever in these two populations, one protected, the other unprotected, or at least that does not protect itself, are entirely different, as we shall now see.

In the following figures, however, we are able to separate the data of the different races, and also we are able to do this for each month over a period of five years. The data are based on the admissions into Ancon Hospital, as in the monthly statistics for the Canal Zone no separation into races is made. The difference between the three races is briefly as follows:

(1) Americans—'gold employees'; intelligent; living in well-kept mosquito-proof houses; use of quinine at onset of fever universal; receive pay when in hospital.

(2) Europeans—Spaniards, Italians and West Indian negroes (!). Those who desire it, live in mosquito-proof houses. Neglect individual prophylaxis; indifferent to personal hygiene; *receive no pay when in hospital.*

(3) Negroes—large majority live in cheap lodging houses, or in huts; personal hygiene entirely lacking.

Now when we consider malaria and blackwater fever in these races, as represented in Chart B, we see brought out very clearly (1) that the incidence of malaria is mainly on the Europeans, much less in the Americans and still less in the Negroes. The seasonal incidence of malaria is shown very clearly in the case of Europeans and Negroes, especially in the latter years. In Americans there appears to be no clear seasonal incidence (of admissions), due no doubt to the fact that 'they universally take quinine at the onset of fever.' (2) It is precisely in the Europeans, who suffer severely from malaria, that we find blackwater, the cases being extremely few in Americans and Negroes.

Now it appears to me that the explanation is obvious, viz., that blackwater depends upon malaria. Of course it is possible to argue that the relationship depends upon the fact that we are dealing with two diseases both inoculated by the mosquito, or that those suffering from malaria are debilitated and so open to the attack of this hypothetical other disease, and even if for argument's sake we were to admit such hypotheses we should still be in the position

that this disease affects those suffering from malaria. But we consider that the obvious explanation is the true one. And again, the figures lend no support to the view that there is a quinine haemoglobinuria distinct from blackwater fever, because, if so, we should expect it in the Americans—the quinine takers.

Examining next the European admissions into Ancon Hospital for 5 years for malaria and blackwater fever, respectively, we get the results shown in Table XVII. *Statistically* there is a small but negative correlation. If now, however, we move the blackwater figures back for 4 months (i.e., make the blackwater follow the malaria 4 months later) the result seen in the third series of figures is got, and now there is a strong *positive* correlation, expressed by $r = + \cdot 50 \pm \cdot 04$ ($r = 1$ implies perfect correlation), tending to show that the two diseases are connected, for not only is there a correlation between the seasonal and secular variations which might occur, and yet not necessarily imply any connection between the two diseases at all, but there is also a coincidence between the magnitude of the oscillations of the two diseases, which, so far as the figures go, suggests that there is a real connection between the two.

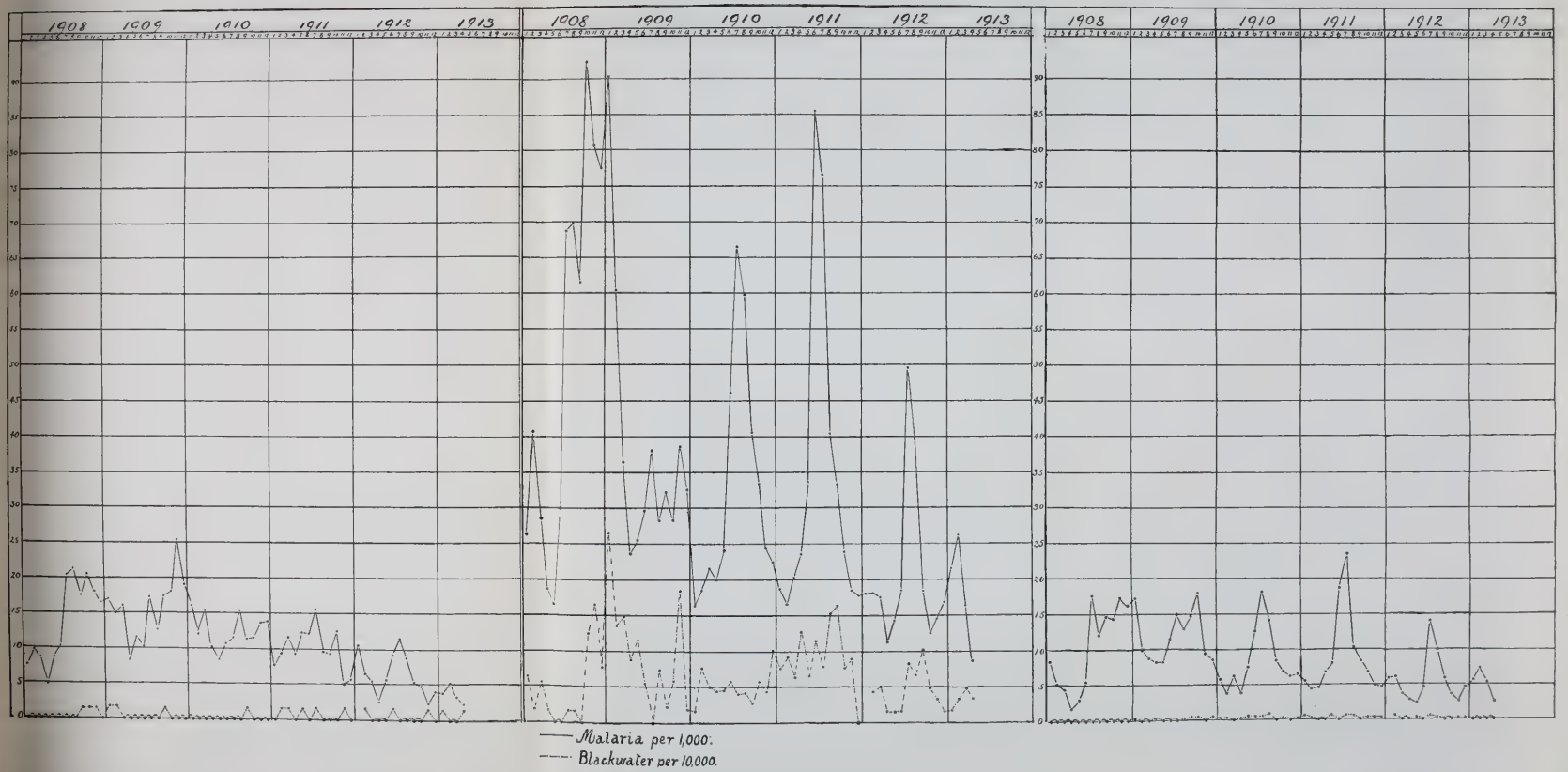
Table XVII.—Europeans, Ancon Hospital

(1) Malaria Cases

Month	1908	1909	1910	1911	1912	5 years	Departure + or − from average 1092
1	123	578	96	122	122	1,041	− 51
2	212	397	96	108	127	940	− 152
3	154	244	129	129	105	761	− 331
4	103	161	136	148	76	624	− 468
5	106	153	161	206	91	717	− 375
6	177	164	317	523	116	1,297	+ 205
7	392	213	499	441	305	1,850	+ 758
8	419	152	431	213	240	1,455	+ 363
9	344	151	298	205	109	1,107	+ 15
10	591	146	233	156	76	1,202	+ 110
11	541	146	168	125	91	1,071	− 21
12	496	172	154	119	103	1,044	− 48
	3,658	2,677	2,718	2,495	1,561	13,109	

CHART B

MALARIA AND BLACKWATER FEVER IN ANCON HOSPITAL.



(2) Blackwater Cases

Month	1908	1909	1910	1911	1912	5 years	Departure + or − from average 20
1	3	17	1	5	2	28	+ 8
2	1	9	4	6	3	23	+ 3
3	3	10	3	4	3	23	+ 3
4	1	6	3	8	1	19	− 1
5	—	7	3	4	1	15	− 5
6	—	3	4	7	1	15	− 5
7	1	0	3	4	5	13	− 7
8	1	4	3	8	4	20	0
9	—	1	2	10	6	19	− 1
10	8	3	4	5	3	23	+ 3
11	11	7	3	6	2	29	+ 9
12	5	1	7	0	1	14	− 6
	34	68	40	67	32	241	

(3) Blackwater Figures Moved Four Months Back

Month	1908	1909	1910	1911	1912	5 years	Departure + or − from average 20
1	—	7	3	4	1	15	− 5
2	—	3	4	7	1	15	− 5
3	1	0	3	4	5	13	− 7
4	1	4	3	8	4	20	0
5	—	1	2	10	6	19	− 1
6	8	3	4	5	3	23	+ 3
7	11	7	3	6	2	29	+ 9
8	5	1	7	0	1	14	− 6
9	17	1	5	2	1	26	+ 6
10	9	4	6	3	2	24	+ 4
11	10	3	4	3	3	23	+ 3
12	6	3	8	1	2	20	0
	68	37	52	53	31	241	

SECOND ATTACKS*

They occur in about 10%. I have not so far examined very extensive records. A very interesting feature about these attacks is that they are most common in the first year, and next so in the first six months. These data are, however, subject to correction for the population in each year period, but, I believe, are approximately correct. Whether this feature indicates increased susceptibility to attack, or whether they are for the most part really relapses, further consideration must show.

TABLE XVIII.—Blackwater Fever: Second attacks.

	6 months	1st year	2nd year	3rd and later years	Total
Graham (1912)	7	15	3	8	26
Christophers & Bentley (1908)	8	12	2	3	17
Deeks & James (1911) ...	8	14	2	—	16
Totals	23	41	7	11	59
Percentages ...	39	69·5	11·9	18·6	

I have to acknowledge the great kindness of Dr. James, Ancon Hospital, Panama, in supplying me with many statistical data and information on many points that arose during a consideration of the Panama figures. I also am indebted to Colonel Gorgas for similar information and interest in my work. To Mr. Stott I am indebted for the great trouble he has taken in examining my figures from the statistical point of view. Finally, I must acknowledge very gratefully the laborious work of Mr. Drawz, Bibliographer to the School, in compiling the data from the Panama Canal Commission Reports and elsewhere.

* By a second attack I mean one that has occurred *after* the discharge of the patient from hospital.

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THE GROWTH AND SPORULATION OF THE BENIGN AND MALIGNANT TERTIAN MALARIAL PARASITES IN THE CULTURE TUBE AND IN THE HUMAN HOST

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PLATES XXXIV, XXXV

CONTENTS

	PAGE
Prefatory note by SIR RONALD ROSS	509
Introduction	510
Cultivation Technique	510
Further observations on cultivation	511
Optimum temperature, Haemolysis, Clumping	512
Resistant forms	513
Morphology of *P. falciparum* in the culture tube	513
,, ,, ,, ,, human host	516
,, ,, *P. vivax* in the culture tube	518
,, ,, ,, ,, human host	519
Summary	520
References	521
Explanation of Plates	522

PREFATORY NOTE BY SIR RONALD ROSS

Researches on the cultivation of the parasites of malaria in Liverpool were commenced some time ago at my suggestion by Dr. Sinton, and then, with better success, by Drs. J. G. Thomson and McLellan, and by Dr. D. Thomson. We are greatly obliged to Sir Edwin Durning-Lawrence, Bart., for giving us the services of Dr. J. G. Thomson for this important enquiry.

RONALD ROSS.

21*st May*, 1913.

INTRODUCTION

The successful cultivation of malarial parasites was first announced by Bass and Johns (1912). Since then several workers, Thomson and McLellan (1912), J. G. Thomson and D. Thomson (1913) and Ziemann (1913) have successfully repeated these cultivation experiments. This achievement has led the way to new discoveries regarding the malarial parasite, and suggests that it may be possible to cultivate *in vitro* any protozoal parasite however specialised it may be.

So far, only the asexual generation of the malarial parasite has been grown *in vitro*. The next step is to cultivate the sexual generation as it occurs in the human host and in the mosquito. We have attempted both, but so far without definite success. We have no doubt, however, that this will also be accomplished sooner or later. In 1911, J. G. Thomson and Sinton successfully cultivated the human trypanosome, and the development they obtained was apparently that which takes place in the stomach of the tsetse fly. Joukoff (1913) states that he has cultivated the mosquito cycle of the malarial parasite, though his results have not yet been confirmed. There is no reason to doubt that the phases of development of protozoa in insects may be produced in the culture tube.

An abridged account of these researches, illustrated by Plate XXXIV, was published in the Proceedings of the Royal Society, B, Vol. 87 (1913), pp. 77-87. We have much pleasure in returning thanks to the Royal Society for permission to reprint Plate XXXIV.

We wish to thank Dr. H. B. Fantham for his very valuable help during our work on this subject.

CULTIVATION TECHNIQUE

Our method of cultivation is almost the same as that of Bass and Johns, except that it is less complicated. Ten c.c. of blood is drawn from a vein and transferred to a sterile test tube containing a thick wire leading to the bottom of the tube from the cotton wool plug. 1/10 c.c. of a 50 per cent. aqueous solution of glucose is added to this tube, preferably before adding the blood.

The blood is defibrinated by gently stirring with the thick wire. Defibrination should be complete in about 5 minutes. The wire with the clot is then removed and the blood is poured into several smaller sterile tubes (about one inch column of blood in each). A rubber cap is placed over the cotton plugs to prevent evaporation and the tubes are then transferred (standing upright) to an incubator at a temperature of 37° to 41° C. The corpuscles settle in a short time, leaving about one half-inch of clear serum at the top. It is apparently unnecessary to remove the leucocytes by centrifugalisation.

FURTHER OBSERVATIONS ON CULTIVATION

We have grown four complete generations of parasites in one tube by the above method, and we do not see why their growth should not continue indefinitely, provided fresh serum and corpuscles be added. It is not the presence of leucocytes which prevents further development. This is due to degenerative changes taking place in the corpuscles and serum. If the serum and corpuscles be kept in a sterile condition in an ice chest they remain unchanged for a long time; but at the temperature required for the growth of the parasites visible changes take place in a few days. The corpuscles become fragmented and form a brownish débris, and the serum becomes dark brown in colour. When this occurs the parasites are unable to continue their developmental cycle. Bass and Johns in their original paper (1912) stated that the parasites grow only on the surface layer of corpuscles, and that no growth took place in the deeper layers. They also stated that the serum destroyed the parasites when they escaped from the corpuscles, so that when the spores escape they must enter immediately into a contiguous corpuscle in order to survive. The blood also, in their opinion, required to be heated to a temperature of 40° C. to destroy the complement in the serum; furthermore, in order to cultivate several generations, the leucocytes had to be removed. We do not believe that they have sufficient evidence for these deductions. We find that the parasites develop even in the very deepest layer of the column of corpuscles. Also, it seems rather a contradiction that the parasites should grow best on the surface layer of corpuscles

next to the serum and the leucocytes which are supposed to destroy them. No doubt the leucocytes ingest some of the parasites, but they are never able to ingest all of them. Again, in one of our most successful cultures the temperature of the blood never exceeded 38° C., and we have found that they are able to grow at a temperature as low as 36° C. This is rather against the theory of complement destruction. In an able paper by Mary Rowley Lawson (1913), considerable evidence is brought forward to show that the parasites are extra-corpuscular during their entire development. If this is true, then the parasites would be constantly in contact with the serum which is supposed to destroy them. It appears to us that the only conditions necessary for the successful cultivation of the parasites are fresh corpuscles, fresh serum, a temperature of 37° to 41° C. and the requisite amount of glucose. The presence of leucocytes and complement is apparently immaterial.

THE OPTIMUM TEMPERATURE

In our opinion the optimum temperature for cultivation is about 38° C. On two occasions we incubated identical culture tubes from the same patient, some at 37° C. and some at 41° C. On both the occasions the parasites developed much more successfully in the tubes incubated at 37° C.

HAEMOLYSIS IN THE CULTURE TUBES

This is a rare occurrence, having been seen only once out of fifteen cases. A kind of haemolysis occurs after the blood has remained in the incubator for several days. The corpuscles degenerate into a brownish débris, and the serum shows a brown discoloration.

CLUMPING OF THE MALIGNANT TERTIAN PARASITES

This phenomenon has been observed in all our malignant tertian cultures (twelve cases). It occurs even when the parasites are scarce. It is best observed in wet films, as smearing of the blood tends to break up the clumps. No tendency to clumping occurred in our benign tertian cultures.

RESISTANT FORMS OF PARASITES

Sometimes it will be found that the parasites do not grow well *in vitro*. They may grow only partially, stopping short of segmentation. This is liable to occur if quinine has been given to the patient before the blood is drawn. In other cases, only a few of the parasites reach maturity, these apparently being able to resist successfully adverse conditions in the culture tube.

THE MORPHOLOGY OF *PLASMODIUM FALCIPARUM* IN CULTURE

The blood examined before incubation always showed the typical small ring parasites of malignant tertian malaria; these varied in size, with a maximum diameter of about 3μ. There was no enlargement of the red blood corpuscles, and no Schüffner's dots. Plate XXXIV, fig. 1, shows a small ring parasite from the peripheral blood before incubation. After many experiments in Liverpool it has been found that there is great variation in the rate of growth of *P. falciparum* in artificial media, and many suggestions can be offered to explain this phenomenon. J. G. Thomson and S. W. McLellan (1912) found in one case that maximum sporulation of *P. falciparum* occurred in twenty-five hours. This culture was made from a case with very heavy infection of parasites, and the patient had taken no quinine. The temperature of the incubator was only 38° C., but the glucose added was slightly in excess of that recommended by Bass (1912). The rapid segmentation of the plasmodia in this case, therefore, may have been due to two causes: (1) the age of the parasites when introduced into the culture tube, since it is to be noted that they were fairly large rings, about 3μ in diameter, and (2) the quantity of glucose may in some way have hastened the growth. In other experiments, however, where the glucose was slightly in excess there was no such rapid growth, so that in all probability the real cause of the rapid segmentation of parasites *in vitro* is due to the age of the plasmodia when drawn for incubation purposes. Another factor which seems to influence the rate of growth is the previous administration of quinine, and, in fact, this may inhibit the growth entirely. On several occasions we have made unsuccessful attempts

to cultivate the malignant tertain parasite, and these have usually been from patients to whom quinine had been administered.

On two occasions complete sporulation did not take place till after fifty hours' incubation at 41° C. On one of these occasions the parasites completed several generations, as illustrated by the accompanying coloured plate (Plate XXXIV, figs. 1-20). We found that maximum segmentation took place in fifty-two hours. Division of the chromatin into daughter nuclei began in about thirty-six hours (figs. 4 and 5). In forty-seven hours the number of spores had increased (figs. 6 and 7), and more or less complete segmentation took place in fifty-two hours. Fig. 11 shows a parasite which has produced thirty-two daughter cells, and these have broken loose from the corpuscle. It is to be noticed that in the peripheral blood the small ring parasites show no pigment. After incubation the rings gradually grow in size (fig. 3), and at a certain stage, immediately previous to the splitting of the chromatin a round compact mass of pigment appears, usually situated near one margin of the parasite, which now measures about 5μ or 6μ in longest diameter. In the culture under discussion this round mass of pigment was seen in thirty-six hours (figs. 4 and 5). J. G. Thomson and S. W. McLellan (1912) found that the pigment had collected in twelve hours. The parasites immediately previous to segmentation measure about 5μ to 6μ in their longest diameter, and all show the circular mass of compact pigment. We may call this stage the pre-segmenting stage. The chromatin now begins to split into two, and if segmentation is complete thirty-two daughter cells may be formed (fig. 11). All stages of segmentation can be found, from two spores up to thirty-two spores as a maximum. During segmentation the circular mass of pigment takes a central position, and the spores form in a circular arrangement around these (figs. 7 to 11). In this culture segmenting forms were found at different stages up to fifty-six hours, this being, no doubt, due to the fact that the parasites were not all of the same age when introduced into the culture tubes.

In seventy-five hours all segmenting forms had disappeared and only very young parasites were found, about 1·5μ in diameter and containing no vacuole (fig. 13). This represents the beginning of a second generation. It is to be concluded, therefore, that many

of the young merozoites escape ingestion by the leucocytes and enter a new red cell. The same culture examined in three days again showed segmenting forms (fig. 14); but in this case the spores were never so numerous as 32, and this can easily be explained by the adverse conditions which have now developed in the culture tube. These conditions did not, however, prevent the young parasites attempting to segment. In four and a half days (fig. 15) we again only found young rings, which represent the beginning of a third generation; and in six days sporulating forms were again found (fig. 16). Here, only eight spores have formed, and it was evident that the power of the parasite to undergo full segmentation was getting gradually less. On the seventh day, again, only very young plasmodia were found, which represented a fourth generation, and these again attempted to sporulate (figs. 18 and 19) on the eighth and ninth days respectively. On the tenth day only young parasites again were found, which represented a fifth generation. No further development, however, occurred, as the conditions in the culture were gradually becoming more and more adverse. Thus we have evidence that the parasites will, under very suitable conditions, actually proceed in the original culture tube through four complete generations without the medium being in any way renewed.

We now wish to draw particular attention to several points of great interest which occur in the cultures of *P. falciparum*. In these there is a definite tendency for the parasites to clump together into masses immediately the circular mass of pigment appears, and even before segmentation begins. A mass of seven parasites is seen in the microphotograph, Plate XXXV, fig. 2. This photograph was taken after twelve hours' incubation at 38° C. In all of these note the dark circular mass of pigment, the parasite having a diameter of about 6μ. At this stage the chromatin is dispersed into the protoplasm of the plasmodium, and as yet shows no division into daughter cells. This tendency to clump becomes more marked when sporulation actually begins, and fig. 3, Plate XXXV, shows a large mass of sporulating parasites, the photograph being taken after twenty-five hours' cultivation. This picture shows all stages of sporulation and illustrates in a very striking manner the tendency of the parasites to agglomerate into masses. This clumping in

malignant tertian malaria was seen by Dr. Cropper (1908) in the peripheral blood of a patient in Jerusalem, and with his kind permission we have been able to reproduce a microphotograph (Plate XXXV, fig. 5) of his plate in the 'Lancet,' which shows a mass of these pre-segmenting parasites, each with a circular mass of pigment. This must have been a most unusual case, as these forms are seldom seen in the peripheral blood in malignant tertian malaria; but it is of extreme interest, since it proves the tendency of the parasites to clump together during the pre-segmenting stage of development. When we compare this figure of Dr. Cropper's, photographed from the peripheral blood of a case of malignant tertian malaria (fig. 5), with our own microphotograph, obtained from a culture twelve hours old (fig. 2), we are at once impressed with this phenomenon; and it is to be noted that this tendency to clump remains until full sporulation. Fig. 3 shows a whole field of a microscopic preparation filled with segmenting forms. In our opinion this phenomenon explains why the sporulation of *P. falciparum* occurs, as a rule, only in the capillaries of the internal organs. All the large parasites tend to get caught in the fine capillaries of the internal organs, and thus only young rings, whose containing corpuscles have not yet acquired the clumping tendency, are seen in the peripheral circulation. This fact explains many phenomena of pernicious malaria, and shows how comatose malaria may occur in infections with *Plasmodium falciparum*, in which condition the brain capillaries are filled with sporulating parasites. Why Dr. Cropper's case showed pre-segmenting forms and also segmenting forms in the peripheral blood is uncertain, but it could well be suggested that these were the overflow from fine capillaries of the internal organs which were already packed with parasites, and could not hold more. It has been observed by us that segmenting forms of *P. falciparum* are only found in the peripheral blood of patients suffering from extremely heavy infections.

THE MORPHOLOGY OF *PLASMODIUM FALCIPARUM* IN THE HUMAN HOST

This is identical in every respect with that obtained *in vitro*, so that it is hardly necessary to describe it in detail. As already stated, only the young ring forms of this parasite are found, as a

rule, in the peripheral blood. This is due to the fact that when these parasites have grown larger than the ring stage, the containing corpuscles stick together, forming clumps which are unable to circulate, and thus are arrested in the fine capillaries of the inner organs. The study of the further stages of this parasite is obtained by examining smears of the inner organs, such as the spleen, liver, brain, etc., of deceased patients. The autopsy smears of over one hundred cases have recently been examined by one of us (D. T.) in conjunction with Dr. W. M. James, in Panama (the results of this work will be published later). In such smears the parasite is found in different stages of development. In some, pre-segmenters only are found; some show only young rings, and others only sporulating forms. This depends entirely on the stage of development of the parasite at the time of the patients' death. In some cases, of course, several stages of development are found, since one patient may contain several broods of parasites which are in different stages of development. In only one case did we find the parasites in the stage of maximum sporulation. All of them contained over 20 spores, and 13 per cent. had 32 spores. One of these is represented in the microphotograph (Plate XXXV, fig. 4). We think that there can be little doubt that we have proved conclusively, both from observations in the culture tube and in the human host, that *P. falciparum* is capable of producing a maximum of 32 spores under favourable circumstances. This is a much larger number than has been previously given in the writings of competent observers. Ross (1912) quotes the figures of Welch (1897) at 6 to 20 or more spores. Later, however, from observations made by himself and one of us, he taught that the maximum number was 2^5 or 32. Marchiafava, Bignami and Mannaberg (1894) state that the numbers vary from 8 to 15, while the following authors give the numbers as follows. Stephens and Christophers (1908) 8 to 10, Deaderick (1910) 5 to 25 and even 30, Gulland and Goodall (1912) 8 to 15. It is probable that these discrepancies are due to two reasons: (*a*) observations of autopsy smears in which the sporulation had not reached its full maturity, and (*b*) observations on autopsy smears of patients to whom quinine had been given before death. In such cases incomplete and atypical sporulation is seen. We do not claim,

of course, that 32 spores are always produced, even under favourable conditions, without quinine administration. It is likely that the numbers produced vary considerably, just as in the case of benign tertian, which produces numbers varying from 16 to 26. The clumping phenomenon in the human host is represented in Plate XXXV, fig. 5. This clump was, as already stated, found by Dr. Cropper in a peripheral blood smear. A similar phenomenon is seen in the brain capillaries of cases of comatose malaria.

THE MORPHOLOGY OF *PLASMODIUM VIVAX* IN CULTURE

Here we are studying a parasite in which all stages, from small rings up to full sporulation, may be seen in the peripheral blood. Sporulation does not necessarily occur in the internal organs, and it is quite usual to find these forms in ordinary peripheral blood smears. When cultivating this parasite, therefore, it is important to draw the blood from the patient when the young forms predominate, so that it is then certain whether or not we are obtaining further development in the culture tubes. In the culture illustrated in Plate XXXIV, figs. 21 to 30, we obtained the blood when young rings (fig. 21) predominated, and no segmenting forms were found. After eight hours' incubation at a temperature of 39° C., a marked increase in size of the parasites was noted, figs. 22, 23 and 24, and pigment was now evident, being scattered throughout the protoplasm in fine granules. This arrangement of the pigment in *P. vivax* is in marked contrast to what occurs in *P. falciparum*, where the pigment always becomes arranged in a dense circular mass from the commencement. After 20 to 29 hours' incubation (figs. 25-30), sporulation was seen at different stages, the pigment being collected into a loose mass of granules in the centre of the parasite. Fig. 29 shows a parasite with 15 daughter cells, and we have found on several occasions sixteen spores, which we think is the usual maximum of daughter cells found in *P. vivax*, although again we find all stages, from two spores up to sixteen, according to the stage of development at which we examine the cultures. We are quite certain that the spores are never so numerous as in the malignant tertian parasite. In these cultures clumping has not been found, and this explains why the parasites do not tend to be

arrested in the internal organs during sporulation, although when full grown they are much larger than the malignant tertian parasite.

The absence of clumping in the case of the benign tertian parasite explains satisfactorily the lack of pernicious symptoms in this infection, and, hence, the absence of comatose malaria, and it also explains why all stages of this parasite are found in the peripheral blood, even up to sporulation.

THE MORPHOLOGY OF THE BENIGN TERTIAN PARASITE IN THE HUMAN HOST

This is identical with that which we have just described under morphology in the culture tubes (Plate XXXIV, figs. 21-30). The chief features in which this parasite differs from *P. falciparum*, are the large size of the containing corpuscle with the presence of Schüffner's dots, the straggling form of the medium-sized parasites, the scattered pigment in the pre-segmenting stages and the smaller number of spores. These spores are larger than the spores of the malignant tertian parasite. The pigment in the sporulating forms is collected into a loose mass. With regard to the number of spores produced by this parasite, we have found as many as twenty-four, but the most usual number is 16 to 18. In culture the largest number we obtained on three occasions was 18. The following are the numbers of spores produced by *P. vivax* in the human host, according to several observers. Ross (1910) quotes the figures of Grassi and Feletti, 15 to 20 spores. Marchiafava, Bignami and Mannaberg (1894), 16 spores. Golgi quotes 14 to 19 spores. Stephens and Christophers (1908), 15 or more spores. Deaderick (1910) gives 12 to 26 spores, most often 16 spores. In our opinion Deaderick's figures are most correct. All stages of this parasite are found in the peripheral blood, and the phenomenon of clumping has never been observed. Ross has taught recently that there are four splits with this parasite—that is, sixteen spores; three splits with the quartan parasites—that is, eight spores; and five splits, or thirty-two spores, with the malignant parasite.

SUMMARY

(1) The malignant tertian parasite has been successfully cultivated, after the method of Bass and Johns, on twelve occasions, and the benign tertian parasite on three occasions by the present writers.

(2) It is unnecessary to remove the leucocytes from the blood before incubation. The optimum temperature would appear to be about 38° C., and the parasites may grow successfully at a temperature of 36° C. or 37° C.

(3) The time required for the full development of the parasite *in vitro* varies, but this variation is partly due to the age of the parasite at the time of incubation.

(4) The cultures of benign tertian differed from those of malignant tertian in that there was no tendency to clumping of the parasites in the former, either before or during sporulation.

(5) This difference appears to us to explain in a satisfactory manner why only young forms of malignant tertian are found in the peripheral blood, as the clumping tendency of the larger forms causes them to be arrested in the finer capillaries of the internal organs. It also explains the tendency to pernicious symptoms, such as coma, in malignant tertian malaria. All stages of the benign tertian parasite are found in the peripheral blood, and there are seldom pernicious symptoms, because there is no tendency to clumping.

(6) The malignant tertian parasite (*P. falciparum*) is capable of producing, in maximum segmentation, thirty-two spores. On the other hand, benign tertian (*P. vivax*) produces, as a rule, during maximum segmentation, sixteen spores; sometimes more may be produced, but the number is never thirty-two.

(7) The pigment in *P. falciparum* collects into a definite, circular, and very compact mass early in the growth of the parasite. On the other hand, during the growth of *P. vivax* the pigment remains scattered in definite granules throughout the body of the parasite, till just before segmentation, when it collects into a loose mass of granules in the centre of the full-grown *Plasmodium*.

(8) The morphology of *P. falciparum* and *P. vivax* in the human host is identical with the morphology of these parasites, as obtained in the culture tube.

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EXPLANATION OF PLATES

Plate XXXIV

All the figures in this plate represent the growth of the parasites in the culture tube. The figures were drawn with an Abbé camera lucida from stained preparations. Magnification 1,600 diameters.

Fig. 1 is a malignant tertian parasite at the time of inoculation of the culture tube. Corpuscle is shrunken. No pigment is seen and no stipulation of the corpuscle.

Fig. 2 represents 12 hours' growth at 36° C. No pigment is yet visible.

Fig. 3 represents 23 hours' growth at 41° C. Pigment is not yet evident, but the parasite has increased in size.

Figs. 4 and 5 represent 36 hours' growth; note the appearance of a compact mass of pigment. Fig. 5 is a double parasite.

Figs. 6 and 7 show commencing segmentation after 47 hours' incubation. Fig. 6 shows five daughter cells, and fig. 7 twenty spores.

Figs. 8, 9 and 10 show sporulating forms after 51 hours' incubation.

Fig. 11 shows complete sporulation (32 spores) after 52 hours' incubation. The containing corpuscle has burst and liberated the spores.

Fig. 12 was obtained after 56 hours' incubation.

Fig. 13 represents a young merozoite of the second generation which has entered a new corpuscle after 75 hours' incubation. Note that there is no vacuole. The parasite is only 1·5μ in its longest diameter.

Fig. 14 represents sporulation of the second generation after 3 days' incubation.

Fig. 15 shows a young merozoite of the third generation after $4\frac{1}{2}$ days' incubation.

Fig. 16 represents sporulation in the third generation after 6 days' incubation. Only eight spores have formed.

Fig. 17 is a young merozoite of the fourth generation after 7 days' incubation.

Fig. 18. Eight days' incubation, showing commencing segmentation in the fourth generation.

Fig. 19. Nine days' incubation, showing sporulation in the fourth generation.

Fig. 20 shows a young merozoite of the fifth generation after 10 days' incubation.

All of the above figures were obtained from one culture tube, without the removal of leucocytes and without the addition of fresh serum or corpuscles.

Fig. 21 shows a young benign tertian parasite at the time of inoculation of the culture tube. The culture was made at the period when these young rings predominated. Note the large size of the corpuscle and the Schüffner's dots.

Figs. 22, 23 and 24 represent 8 hours' growth in culture tube at 39° C. The parasites have now distinctly increased in size, and fine granules of pigment are now seen scattered in the protoplasm.

Figs. 25-30 show parasites obtained from the culture tube after 20 to 29 hours' incubation. Fig. 25 shows four masses of chromatin.

Fig. 30 is a female gamete (undivided chromatin and scattered pigment) found in the culture tube; similar gametes were found in the blood at the time of inoculation of cultures, so it is probable that this gamete was one of these and that it had not developed in the culture.

Note the large size of the corpuscles in the benign tertian as compared with those in the malignant tertian; also the Schüffner's dots and the scattered pigment. The spores also are larger and fewer in number in the case of the malignant tertian parasites.

Plate XXXV

Microphotographs of malignant tertian parasite. Figs. 1 to 3 are from the same culture of *Plasmodium falciparum*, and are all photographed at the same magnification, namely, 1,500 diameters.

Fig. 1. A photograph of the malignant tertian parasite as seen in the peripheral blood before incubation. No pigment is seen. The small dark mass is the chromatin.

Fig. 2. The malignant tertian parasite after 12 hours' incubation at a temperature of 37° C. to 38° C. The parasites have grown to at least twice the size. A circular mass of compact pigment is now evident. This photograph illustrates the clumping tendency and seven parasites are seen grouped together. No division into daughter cells has yet taken place. The dark circular area is pigment, and the darker area, towards the margins, is due to the chromatin.

Fig. 3. The malignant tertian parasite after 25 hours' incubation at a temparature of 37° C. to 38° C. This photograph shows well the tendency to clump and the parasites are now seen at all stages of sporulation.

Fig. 4. Shows two parasites from a smear of the spleen showing segmentation. Thirty-two spores can be counted in the one in the centre of the field.

Fig. 5. This is a photograph taken from a coloured plate drawn by Dr. Cropper and shows the clumping of the red cells containing pre-segmenting forms of the malignant tertian parasites. This drawing was made from a smear of the peripheral blood (*vide* 'Lancet,' July 4th, 1908, and coloured plate fig. 1). (Magnification about 1,000 diameters).

THE TRICHONOCARDIASES

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PLATES XXXVI—XXXVII

INTRODUCTORY

The literature relating to the Trichonocardiases being of such recent date and of such small bulk, together with the fact that this is the first record of their occurrence in the Anglo-Egyptian Sudan, and, as far as we know, in Africa, has induced us to bring forward the following observations.

HISTORICAL

A number of affections of hairs have been described which are characterized by the presence of variously coloured nodes, nodosities and more or less elongated unilateral, bilateral or annular thickenings on hairs in various parts of the body. The nodes are confined to the shaft, and do not penetrate into the hair follicle. These affections may be classified as the Trichosporoses, Lepothrix and the Trichonocardiases.

Trichosporosis was first described as a rare disease of the hair of the head in native women living in the valleys of the province of Cauca on the slopes of the Eastern Cordilleras of Colombia. It has been observed in Rio de Janeiro and Germany. It also occurs on the hair of the beard in men. Osorio gave it the name 'Piedra,' because of the stony hardness of the nodules, but the more correct name is Trichosporosis tropica, as its causal organism is *Trichosporum giganteum*, Behrend, 1890. A similar condition has been

briefly described by Castellani as occurring in India and Ceylon, but so far, he has not named the fungus, which appears to differ from *T. giganteum.*

The remaining Trichosporoses belong to temperate climates; they are:—

Biegel's Trichosporosis, or Tinea nodosa of Cheadle and Morris, due to *Trichosporum beigeli* (Rabenhorst, 1867). It was first found by Lindemann in 1865, and is often called the Chignon fungus. It occurs on the hair of the head.

Unna's Trichosporosis, or Piedra nostras, found on the hairs of the beard and moustache, and due to *T. ovale* (Unna, 1896).

Behrend's Trichosporosis, or Nodular trichomycosis, found on the hairs of the beard, and due to *T. ovoïdes* (Behrend, 1890).

Du Bois' Trichosporosis, found on the hairs of the pubic region, and due to *T. glycophile* (Du Bois, 1910).

Another nodular hair disease is Lepothrix, or scaly hair, which was discovered by Paxton in 1869, was named by Wilson, and described by Eisner and Crocker. It is characterized by variously coloured grey, yellow, or brown nodules, which surround the hairs of persons who perspire profusely. The hairs most usually attacked are those of the axillae, but the disease may also be found on those of the chest, pubes, and inside of the thighs. It is common in Germany, and is believed to be due to a diplococcus forming zoogloea masses. Allied to Lepothrix is the affection called Trichomycosis capillitii by Winternitz in 1903, which is said to be due to a straight or slightly bent bacillus. The descriptions of these conditions suggest that there may be some confusion, and that in reality they may be Trichonocardiases in which either only the diplococcus has been observed or in which only the bacilliform fungus has been seen. In any case it would seem that the disease Lepothrix requires further investigation, especially as Crocker's excellent description closely agrees with Trichonocardiasis.

The Trichonocardiases are allied in appearance to Lepothrix, but differ therefrom in the fact that they are all due to a fungus *Nocardia tenuis* (Castellani, 1911), either alone as in Trichonocardiasis flava (Pl. XXXVI, figs. 1 and 2; Pl. XXXVII, fig. 13), or in association with a Micrococcus as in Trichonocardiasis nigra (Pl. XXXVI, figs. 4 and 5; Pl. XXXVII, figs. 14, 15 and 20), and

T. rubra (Pl. XXXVI, figs. 6 and 7; Pl. XXXVII, figs. 16, 19 and 22). They were first described by Castellani in 1911 in a paper published in the British Journal of Dermatology, and this description was extended by the same author in 1912 in an article published in the Transactions of the Royal Society of Medicine, and still later, in 1913, in the second edition of the Manual of Tropical Medicine published by himself and one of us. He named the disease Trichomycosis axillaris, and sub-divided it into three varieties, flava, rubra and nigra; but as it occurs on the hairs of the pubes, in some cases without any axillary infection, we are of the opinion that Trichonocardiasis is a better term.

Attention has, very briefly, been drawn to Castellani's work by Jackson and McMurty in their book on 'Diseases of the Hair,' published in 1913, where they devote a small section to the subject under the title 'A Disease resembling Lepothrix.'

So far as we know, the above comprise the total literature on the subject of the Trichonocardiases.

GEOGRAPHICAL DISTRIBUTION

We have found the three varieties of Trichonocardiasis in the Anglo-Egyptian Sudan, and one of us has observed a condition resembling Trichonocardiasis flava in Europeans on the Gold Coast, West Africa, as long ago as 1898.

As the three varieties may be found on one and the same person, and, indeed, in the same axilla, we do not propose to give separate descriptions of each variety, but to describe the disease Trichonocardiasis as a whole, mentioning the difference between the three varieties when necessary.

At present the Trichonocardiases are only known to exist in the Tropics, i.e., in Ceylon, the Anglo-Egyptian Sudan, and on the Gold Coast. These regions being so widely separate one from the other, it is reasonable to suppose that the disease will be found in other tropical regions when looked for.

Castellani has drawn special attention to the fact that he found his cases in the hot, damp districts of Ceylon, and adds that it will probably be found occurring in every low-lying tropical region. While our Gold Coast cases support Castellani's views, the cases we

have found in Khartoum demonstrate that the disease may also occur in a hot and dry climate and at a certain elevation. We found our first cases here in the month of May of this year, when the highest shade temperature was 112·2° F. (44·5° C.), and the mean shade temperature was 105·9° F. (41° C.). During this time the least percentage humidity of the air was 4% and the average 21%, while the evaporation from Piche's tube rose as high as 30·1 millimetres. As the elevation of Khartoum is 390 metres above sea-level at Alexandria, it is not low-lying. We, however, agree with Castellani that the disease will probably be found in many tropical regions, low-lying or elevated, if looked for.

RACIAL DISTRIBUTION

Most of our cases have occurred in Europeans, but Castellani has seen it among the Sinhalese and we have met with it in a negro. Possibly its occurrence was noticed long ago by native doctors, and may be one reason why so many native races shave the axillae.

BODY DISTRIBUTION

So far we have only seen the infection on the hairs of the axillae, pubic regions, and folds of the groin and in this we are entirely in agreement with Castellani.

The reason for the distribution appears to us to be the nutrition of the causal fungus *Nocardia tenuis*, as it would appear to require a mixture of human proteid material such as is found in blood serum and normal saline before it will sprout. As we shall point out later, it derives its nutriment from the lymph of the cortex of the hair while at the same time it is bathed by the saline sweat of the axillae, pubic regions, and groins, and this appears to be the reason of its affinity for these particular parts of the body.

AETIOLOGY

The causal agent is *Nocardia tenuis* (Castellani, 1911) (Pl. XXXVI, fig. 9; Pl. XXXVII, fig. 17), either alone as in the variety Trichonocardiasis flava, or associated with *Micrococcus nigrescens* (Castellani, 1911) as in Trichonocardiasis nigra, or with *Micrococcus castellanii* (Chalmers and O'Farrell, 1913) (Pl. XXXVII, fig. 18) as in Trichonocardiasis rubra.

We will now consider these aetiological factors seriatim.

NOCARDIA TENUIS (Castellani, 1911). This organism can be readily seen in cleared specimens of the infected hairs mounted in gelatin or Canada balsam as minute rod-like or bacillary forms often lying more or less parallel to one another and embedded in an amorphous ground substance (Pl. XXXVII, fig. 22), probably excreted by the fungus. This ground substance, as will be explained later, is fixed to the cortex of the hair, and lies under the cuticle and some of the superficial layers of the cortex.

When individual hyphae are examined after coloration by any of the ordinary stains, they are seen to be narrow, elongated, unbranched, non-septate rods resembling bacilli (Pl. XXXVI, fig. 9; Pl. XXXVII, fig. 17). They may be short or long, straight or slightly curved, but they are always fairly and usually very narrow.

A series of measurements gives the average length as varying from about 2·0 to over 7·0μ, and the average breadth from 0·14 to 0·3μ.

Each hypha is enclosed in a cell wall, inside which lies the cytoplasm, which may stain very lightly with the colouring agent used, but may also stain fairly darkly, especially in certain areas. No nucleus can be demonstrated and the cytoplasm appears to be homogeneous except at certain places where well-defined deeply staining rounded granules may be seen (Pl. XXXVI, fig. 9). These granules, which measure about 0·14 to 0·3μ in transverse diameter, may be situated at one end of the hypha, as in Pl. XXXVI, figs. 9, 10, 12, and Pl. XXXVII, fig. 21, or this may be placed at regular intervals through the cytoplasm (Pl. XXXVI, fig. 9), or one may be at one end while others may be close together near the opposite pole. We have not met with any branched forms, except in cultivation, but we have seen forms with one end rather bulbous, resembling in miniature the club forms of some other Nocardias. We have not succeeded in cultivating this fungus permanently, in bulk, or pure cultivation, but we have succeeded in inducing growth by placing scrapings from the hairs in equal parts of human blood serum and 0·85% normal saline solution in hanging drop preparations. These were first kept for twenty-four hours at the room temperature, which varied from 90° F. (32·2° C.) at night to about 105° F. (40·6° C.) in the day, and were then placed in an incubator at 104° F. (40° C.).

Under these conditions the fungus quickly grew out of its amorphous bed (Pl. XXXVI, fig. 10) into the surrounding liquid, and proceeded to develop in the manner depicted in Pl. XXXVI, fig. 12, and Pl. XXXVII, fig. 21, the latter of which is a microphotograph.

It will be noticed that the branching is monopodial, and that the hyphae are non-septate.

With regard to its position in the vegetable kingdom it is obvious that, as it possesses neither chlorophyll nor chromatophores, it must belong to the class Fungi, and that it belongs to the fungal order Deuteromycetes or Fungi imperfecti, because its life-cycle is unknown. Further, it can be placed in the sub-order Hyphomycetes because perithecia, asci and immersed stroma are also unknown. Of the families of the Hyphomycetes it belongs to that of the Mucedineae because its hyphae are pallid and do not cohere to form a compound stem-like structure. Lastly, it may be grouped with other forms in the genus *Nocardia* (Toni and Trevisan, 1899), which is defined as consisting of mycelial filaments of various sizes, but generally very thin (one micron or less in diameter), often branching, non-septate, and without differentiated nuclei.

Nocardia tenuis (Castellani, 1911) may therefore be defined as follows:—

Nocardia composed of thin, bacillary-like hyphae, varying from about 2 to over 7μ in length, and from 0·14 to 0·3μ in breadth, embedded in an amorphous ground substance attached to the cortex of the axillary and pubic hairs of man in tropical countries.

MICROCOCCUS NIGRESCENS (Castellani, 1911). This coccus has been grown in pure culture by Castellani, who has described its biological characters on page 1521, and has illustrated its cultural appearances in fig. 8 on Plate V of the second edition of the Manual of Tropical Medicine mentioned above. It is therefore unnecessary for us to go into details with regard to this organism, except to say that Trichonocardiasis nigra appears to be much rarer here than the flava or rubra, while the pigmentation on the hairs and in the growths we have obtained is relatively scanty, and we have found it difficult to isolate and grow *Micrococcus castellanii* (Chalmers and O'Farrell, 1913). Castellani in Ceylon found that it was more difficult to isolate and grow this coccus than was the case with *Micrococcus nigrescens*. On the other hand, we,

in Khartoum, have found quite the reverse: we have found it exceedingly difficult to obtain the black coccus with anything beyond a trace of black pigment, while the red coccus grows easily and well.

It is very readily separated out by sowing scrapings from the hair, or pieces of the hair on sloped agar, and picking out either the red colonies which require some time to develop the reddish pigment, or the yellow colonies which always appear first and in which the red pigment subsequently shows itself.

MICROCOCCUS CASTELLANII is a round or oval coccus, measuring from about 0·3 to about 0·7μ in diameter (Pl. XXXVII, fig. 18). It is divided medianly by a clear central line into two half-moon shaped segments, thus producing the well-known diplococcal appearance. The cocci, which may occur singly or in groups, are colourless and non-motile, but excrete an amorphous non-granular lemon chrome-coloured pigment (classified according to Ridgway's Colour Standards), which lies between the individual cocci.

In old cultures another pigment of dark brownish-red colour appears (according to Ridgway's Standards this colour is madder-brown), but its relationship to the earlier yellow pigment can easily be proved by removing some of it and suspending in a sufficiency of distilled water, when the fluid at once resembles a similar suspension of the yellow pigment. If, however, this is done in a very small quantity of water, a faint reddish tinge can be observed. The best medium for showing the striking yellow pigment is the potato, when in twenty-four hours the growth assumes the appearances depicted on Pl. XXXVI, fig. 11. In order to demonstrate the red pigment it is necessary to cultivate the microccocus for some time. The medium on which this pigment shows best is the ordinary agar slope, which, when old, exhibits the dark-red pigment in the centre and the yellow pigment at the sides (Pl. XXXVI, fig. 8). The yellow pigment is seen both in aërobic and anaërobic growths. In broth and peptone water or liquid blood serum, whether grown aërobically or anaërobically, the pigment is not visible until a very abundant growth appears, when the yellow colour can be seen.

The coccus colours with all the ordinary staining re-agents, and as a rule is decidedly Gram positive. It must, however, be admitted that even in preparations showing most of the cocci well coloured

when treated by Gram's methods, a few may be seen quite decolourised, and we have also seen cocci with one demi-lunar segment well stained while the other was completely decolourised. We have failed to demonstrate a capsule around the coccus.

The organism grows aërobically and also anaërobically. The optimum temperature appears to be 37° C., but it can also be cultivated at the room temperature, which in Khartoum at this season of the year is seldom less than 32·2° C. It also grows at 20° C. on agar slopes, but not as abundantly as at 37° C., and is therefore less pigmented. Its rate of growth depends somewhat upon the medium, as it grows quickest and best on potato, and next best on ordinary or glycerin agar. On solid media it gives rise to a yellow growth at first; but on most agar media, if kept long enough, some red coloration will subsequently be found. The best medium for the red coloration appears to be the ordinary agar slope. With regard to the other agar media, it grows well on glucose and maltose agar. Like *Micrococcus nigrescens*, it produces neither acid nor gas in the following media:—

Peptone-sugars: Glucose, Laevulose, Galactose, Arabinose, Maltose, Lactose, Saccharose, and Raffinose.

Peptone-carbohydrates: Dextrin and Inulin.

Peptone-glucoside: Amygdalin.

Peptone-alcohols: Erythrite, Adonite, Dulcite, Isodulcite, Mannite, Sorbite, and Inosite.

It grows slowly on blood serum, which it does not liquefy, and well in broth and peptone water, in which it forms a general turbidity. It does not produce indol.

In agar stabs the growth is confined to the line of puncture and to the formation of a small yellow knob on the surface, in other words, the growth is filiform.

With regard to its systematic position in the family *Coccaceae* (Zopf, emended Migula), it must certainly be grouped with the genus *Micrococcus* (Hallier, 1866, emended Cohn, 1872).

In this genus it certainly belongs to those forms which grow well on agar media and are Gram positive, and in this division it belongs to the sub-division which produces colours.

The cocci of this sub-division which possess red coloration are

only three in number, *Micrococcus roseus* (Bumm, 1885), *Micrococcus ruber* (Trommsdorff, 1904), and *Micrococcus rubidus* (Hefferan).

Under the term *M. roseus* (Bumm, emended Lehmann and Neumann) are gathered a large number of rose coloured diplococci which are not known to be parasitic, and which produce growths on potato which are limited to the streaks. These growths are faint rose red in colour within an oily lustre, and are often surrounded by a whitish glistening zone, thus giving rise to a very different appearance from that produced by *M. castellanii*. *Micrococcus ruber* (Tromsdorff, 1904) or, as it is sometimes named, *M. chromidrogenus ruber*, which was isolated from axillary hairs, is characterised by the fact that it does not grow on potato, its colouring matter is not soluble in water, and when treated by sulphuric acid the red colour turns blue-green, while *M. castellanii* does grow on potato and its colouring matter is unaffected by 25 per cent. sulphuric acid. Moreover, *M. ruber* is associated with chromidrosis, while *M. castellanii* is not. With regard to *Micrococcus rubidus*, it is impossible to obtain any information as to its characters in Khartoum, but it appears to be closely related to *M. roseus* var *carneus*, and to be non-parasitic.

We, therefore, are of the opinion that the organism which we are describing is not the same as any species of Micrococcus so far named, and therefore give it the appellation *Micrococcus castellanii*, Chalmers and O'Farrell, 1913.

METHOD OF INFECTION

There is no doubt in our minds that the usual method of infection is from man to man, and we base this opinion upon the following observation:—

A young European, known to be quite uninfected with these parasites, became greatly interested in our work; he examined the cases which came to the laboratory, and handled and examined the fresh hairs removed from the axillae.

When he visited the laboratory he was clad in tennis attire, and as he moved about, his shirt rucked up from below repeatedly, and whenever this happened he adjusted it by pulling forward his leather belt with one hand while he pushed the soft shirt downwards inside his trousers with the other. Two weeks after examining the

cases he first noticed a reddish appearance on the hairs of the pubes. On examination it was found that the hairs were infected with the red variety of Trichonocardiasis, that is to say, with the variety which he saw and handled in abundance. It grew rapidly on the pubic hairs, but did not extend to the axillae. Moreover, it only grew in the fold of the left groin, where sweat is apt to accumulate. It did not spread to the right groin.

INCUBATION PERIOD

It would therefore appear as though two weeks were required to elapse before an infection produces sufficient growth on the hairs to attract attention, even when it is being looked for.

PATHOLOGY

The pathology of the infection appears to be quite simple. The fungus arriving upon the hair grows and pushes its way at first under a cuticular scale and then quickly works its passage into the cortex, raising, in so doing, its superficial fibres, which, together with the cuticular scale, form a covering or protection for the fungus (Pl. XXXVII, fig. 19), which probably finds the nutriment it requires in the fluids of the cortex of the hair. Once established in position the fungus does not penetrate deeper into the cortex, but, on the contrary, grows outwards, forming the nodosities, rings and sheaths to the hair which have already been mentioned. It is owing to this method of growth that the hair is so little affected, as the deeper layers of the cortex, the medulla and the root of the hair are not involved in our cases.

MICROSCOPICAL ANATOMY

In an early stage of the infection the cuticle and some fibres of the cortex (Pl. XXXVII, fig. 13, shows this stage at three places between the large clumps of fungus) may be seen to be raised in ridges, which run in wavy lines across the shaft of the hair transversely to its long axis. Under these ridges masses of the fungus may be seen growing.

A parasitic mass is composed of a firm homogeneous ground substance, in which the rod-like hyphae of the *Nocardia* may be seen

(Pl. XXXVII, fig. 22). In this the cocci are also found which are responsible for the coloration of the black and red Trichonocardiases.

SYMPTOMATOLOGY

As already stated the shortest incubation period with which we are acquainted is two weeks. The infection causes no general symptoms and is only discovered by accident by the patient, who then seeks advice in order to know what is taking place. Very often the infection is first met with while the patient is being examined by his medical attendant for some other disease. In regiments it is usually found during a general medical inspection.

The regions of the body which are affected are the axillae and the pubes, most commonly the former alone or in conjunction with the latter, and less commonly only the latter. The hairs of these regions may be affected with one or more of the three varieties already mentioned.

On examining with the naked eye an axilla affected by the yellow variety, it will be noticed that the hairs are covered with a thick or thin yellow deposit (Pl. XXXVI, fig. 1), as though the natural grease had accumulated, and, indeed, this is the reason why some persons ask about the complaint, as they consider that it reflects upon their habits. If the black variety is present it will appear as black or very dark patches, short or long, thick or thin, on one side of or ensheathing the hair (Pl. XXXVI, fig. 5), and the same description holds good for the red variety (Pl. XXXVI, fig. 7), with the exception of the difference in colour. These observations are easily confirmed by microscopical examination, when the masses may be seen encircling the hair, lying on both sides or merely forming excrescences on one side of the shaft. The cuticle and fibres of the cortex may be seen covering the small masses or embedded in the large ones.

As already stated, the disease is confined to the shaft of the hair, the health of which it does not affect. The infection of the hair does not appear in any way associated with excessive sweating, though, of course, the amount of this excretion produced by the axillae is considerably greater in the tropics than in the temperate zone, and, therefore, in warm climates the axillae are generally

damp and the pubic regions are also damper than usual. Probably it is the dampness of these areas of the body which determines the distribution of the infection, as it has so far never been seen on the hairs of the head, face, arms and legs.

The infection dies away on return to a temperate climate, especially in winter.

DIAGNOSIS

The appearance of minute nodules, rings or sheaths of a yellow, black or red colour on the hairs of the axillae or pubes indicates the presence of either Lepothrix or Trichonocardiasis.

The latter may be differentiated from the former by demonstrating the presence of *Nocardia tenuis*, Castellani, 1911, which is absent in Lepothrix.

From Trichosporosis tropica or Piedra it may be distinguished by the fact that it does not attack the hair of the head or face, and also by the difference in the causal parasite. From the other Trichosporoses it may be separated by its distribution and by the presence of *Nocardia tenuis*, as well as by the absence of a Trichosporum.

PROGNOSIS

It appears to have a marked tendency to remain in the region or regions which it first invaded, and does not, as far as our observations go, tend to spread over the body.

TREATMENT

We have found the treatment recommended by Castellani to be quite useful, viz., a lotion of one drachm of formalin to one ounce of water applied twice daily to the affected hairs, while a 2% sulphur ointment is used at night.

PROPHYLAXIS

The infection appears to be only mildly contagious; still it does tend to spread in communities. Cases which are likely to be sources of infection should have their clothes disinfected before being washed. During the infection the underclothing should be dusted with some antiseptic powder, e.g., dermatol, one drachm in one ounce of venetian talc, or with a boracic acid dusting powder.

ACKNOWLEDGMENTS

We wish to acknowledge with many thanks the kindness received from Major Forrest, R.A.M.C., during the preparation of this paper, and the skill with which our artist, Mr. A. Marshall, Senior Laboratory Assistant in these laboratories, has prepared the illustrations.

KHARTOUM,
September 15th, 1913.

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EXPLANATION OF PLATES.

PLATE XXXVI

Fig. 1. Trichonocardiasis flava. Natural size.

Fig. 2. Trichonocardiasis flava. × 60.

Fig. 3. Trichonocardiasis flava. × 40.

Fig. 4. Trichonocardiasis nigra. × 60.

Fig. 5. Trichonocardiasis nigra. Natural size.

Fig. 6. Trichonocardiasis rubra. × 20.

Fig. 7. Trichonocardiasis rubra. Natural size.

Fig. 8. *Micrococcus castellanii;* old culture on an agar slope. Natural size.

Fig. 9. *Nocardia tenuis*; separate hyphae. × 1,200.

Fig. 10. *Nocardia tenuis* sprouting out from the ground-substance; hanging drop preparation. × 1,500.

Fig. 11. *Micrococcus castellanii*; 24 hours' growth on potato. Natural size.

Fig. 12. *Nocardia tenuis*; growing in hanging drop preparation. × 1,500.

Plate XXXVII

Microphotographs of the Trichonocardiases.

Fig. 13. Trichonocardiasis flava. × 60.

Fig. 14. Trichonocardiasis nigra. × 60.

Fig. 15. Trichonocardiasis nigra. × 60.

Fig. 16. Trichonocardiasis rubra. × 60.

Fig. 17. *Nocardia tenuis*, Castellani, 1911. × 1,200.

Fig. 18. *Micrococcus castellanii*, Chalmers and O'Farrell, 1913. × 1,500.

Fig. 19. Hair showing the fungal growth elevating the cuticle and the superficial fibres of the cortex. × 500.

Fig. 20. Trichonocardiasis nigra. × 60.

Fig. 21. *Nocardia tenuis* growing in a hanging drop preparation. × 1,200.

Fig. 22. Fresh preparation of a hair affected with Trichonocardiasis rubra, showing the radiating hyphae at the periphery and the optical section of the hyphae in the centre. × 700.

EPIDEMIC TRICHONOCARDIASIS

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INTRODUCTORY

The literature connected with Trichonocardiasis being new and of small extent, it may be of interest to record an epidemic which recently took place in the Welsh Regiment in Khartoum.

Before commencing the account of the epidemic we may mention that we have heard of a case of Trichonocardiasis rubra in a European while in Aden, but whether it originated there or was merely imported we are not in a position to state.

It would, however, appear that the Trichonocardiases are widely distributed throughout the tropics and, further, that they may occur in epidemics.

Full accounts of the history, aetiology, etc., having been already given during this year by Castellani, O'Farrell and one of us, we restrict our remarks entirely to the present epidemic, its probable origin and its suppression.

THE EPIDEMIC

About the middle of September, 1913, a Private in the Welsh Regiment, which had been stationed in Khartoum for some months, applied for medical treatment on account of irritation of the skin in both axillae. On investigation it was found that the hairs of these regions were heavily infected with Trichonocardiasis rubra. Figure I is a photograph of one of his axillae and shows the remarkably heavy infection of the

hairs which were matted together. The skin surrounding this mass of hairs was red, congested, and very irritable. The individual hairs, separated out and photographed, are depicted, natural size, in Figure II, which is improved if examined by a lens. As, however, this affection of the hair has been recently described by Castellani and by one of us in conjunction with Captain O'Farrell, R.A.M.C., no further remarks will be made as to the clinical appearances or the microscopical findings, except to state that they entirely agree with those already reported.

FIG. I.—Photograph of an axilla, showing infection with Trichonocardiasis rubra. Note the thickenings and the matting of the hairs.

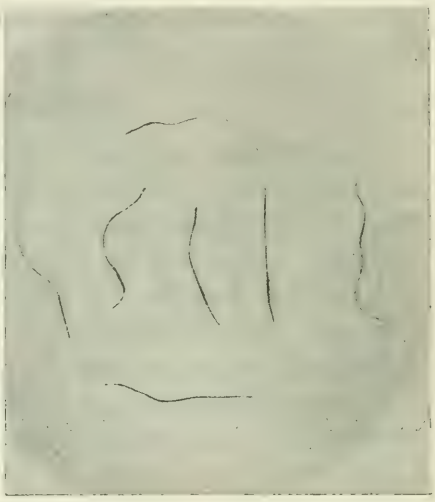

FIG. II.—Photograph of hairs from the axilla depicted in Fig. I, natural size. This figure is improved if examined by means of a lens.

The occurrence of this case led to a systematic investigation of the regiment, when no less than 42 cases, in all, were discovered. Some of the men complained of slight irritation in the axillae, and these men invariably had an area of erythema surrounding the hairs of the axilla, but the majority of men had felt no symptoms and did not appear to have noticed the altered colour of the hair. None of the cases showed any affection of the hairs on the pubis or any other part of the body.

The most common variety was Trichonocardiasis flava, while a

number of cases showed Trichonocardiasis rubra, but no Trichonocardiasis nigra could be found.

The erythema noted was only seen in men suffering from Trichonocardiasis rubra.

A thorough investigation was made as to the possible source of infection, and very early suspicion was aroused that the washermen might possibly be the spreaders.

There were, in all, five washermen and four washerwomen for the cleansing of the regimental clothing. All these, save one, worked in the central washhouse and these were found to be free from Trichonocardiasis. The remaining washerman, who was occasionally assisted by one of the other washermen, went from bungalow to bungalow in the barracks for the purpose of doing extra washing for the men, as the regulation washing was insufficient to keep the clothing clean in a hot dusty country like the Anglo-Egyptian Sudan. This man washed, in particular, the shirts and the khaki trousers for the men. He was found to be infected with Trichonocardiasis flava and rubra, and he was strongly suspected to be the spreader of the infection, as it was not confined to the men of any one company but scattered through all the companies of the regiment.

This washerman and the infected men were duly treated, with the result that during the last inspection, made a few days ago, no new cases were found.

TREATMENT

As so many men were affected it afforded ample opportunity for observing the results of various forms of anti-parasitic treatment.

Most of the cases were treated by means of a lotion of formalin 40 per cent., one drachm to six ounces of rectified spirit. This lotion was carefully applied to the hair twice daily. It was advisable to restrict this application entirely to the hairs as it was often found to irritate the skin if it was carelessly rubbed into the axilla.

At night a 2 per cent. sulphur ointment was applied, thus completing the routine recommended by Castellani. The result was excellent, but it required time, and, on an average, a cure was not effected under three to four weeks.

If the cases did not improve under this treatment, application of tincture of iodine effected a cure in three to four days.

Tincture of iodine alone was also applied, but with doubtful advantage.

Perchloride of mercury was also tried, but it required three to four weeks to bring about a cure.

The best remedy in our hands appeared to be a combination of the formalin-sulphur method for about two days, followed by tincture of iodine, when a cure was effected in several cases in less than a week.

With regard to the erythematous areas mentioned above, these were readily healed by the application of Calamine lotion, associated with the anti-parasitic treatment to the hairs, as already described.

In addition, all the underclothing was dusted regularly with a boric acid powder of the strength of one drachm to the ounce of starch powder. This is not the best powder for the purpose, but it is the cheapest and is readily available. The use of dermatol, europhen or of xeroform with Venetian talc would be better, but the boric acid served its purpose quite well.

KHARTOUM,
November 12th, 1913.

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HEREDITARY INFECTION, WITH SPECIAL REFERENCE TO ITS OCCURRENCE IN *HYALOMMA AEGYPTIUM* INFECTED WITH *CRITHIDIA HYALOMMAE*

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PLATES XXXVIII—XL

INTRODUCTION

Our knowledge of the rôle played by the blood-sucking parasites as transmitters of human and animal diseases has considerably advanced during recent years. Consequently, the study of these vehicles of transmission has increased, and many cases of hyper-parasitism have been discovered. During the search for the pathogenic varieties others have been encountered, and these, though their pathogenicity be confined only to their insect hosts, must not be overlooked.

In three ways, a comprehensive study of their life cycles is of value: Firstly, in order to prevent their stages from being confused with the stages in the life histories of more important parasites. Secondly, to act as a guide to the possible behaviour of the disease-producing varieties, and thirdly, as a weapon of offence against their parasitic hosts.

Probably the most important stage in the life cycle of all hyper-parasites is the hereditary infection stage. Hereditary infection does not deal with individuals but with generations, and this method of infection is a higher grade of organisation and of evolution than simpler methods of infection. The complete life cycles of but few hereditary infections are known, though a considerable number of hereditary infection-transmissions are known to exist.

Consequently any addition to our knowledge of such infections is of value. Moreover, any example that can be studied with any degree of facility is worthy of note. The hereditary infection of *Hyalomma aegyptium* by *Crithidia hyalommae* is one of these, and will be described in the following pages.

HISTORICAL

The occurrence of hereditary infection has been suggested, observed and worked out in the insect flagellates already, and among the authors who advanced this line of research I have primarily to mention Miss A. Porter, whose work on *Crithidia melophagia*, Flu, 1908, has proved of valuable assistance. As early as 1903 Dr. L. W. Sambon put forward the hypothesis of the possibility of the occurrence of hereditary infection of the tsetse fly by *Trypanosoma gambiense*.

P. C. Flu (1908) published an account of a flagellate parasitic in the ovaries and small larval forms of the sheep-ked, *Melophagus ovinus*, Linnaeus.

L. D. Swingle (1909) working in Nebraska, described rounded and plasmodial stages of the same parasite, occurring in the egg of *Melophagus ovinus*.

A. Porter (1910) described the intestinal contaminative method of infection and the full cycle and mode of hereditary infection of the same parasite in the host, egg, developing embryo, and fully hatched *Melophagus*.

In a preliminary note published in August, 1913, I have given a brief description of the flagellate stage and discussed the movements of *Crithidia hyalommae*, O'Farrell, 1913. The movements will not therefore be further described, except the special movement connected with the entry of the flagellate into the ovarian system of the tick. The life cycle passed in the larva and nymph will form the subject of a future communication.

THEORETICAL APPLICATIONS

Apart from the practical proofs of the hereditary transmission of *Crithidia hyalommae* in *Hyalomma aegyptium*, certain theoretical conclusions formed the basis of the search for such proofs. These conclusions were:—

1. *The infection was not inoculative*, i.e., derived from the vertebrate host, because:—

   (*a*) All the ticks collected from one given cow were not infected.

   (*b*) Ticks other than *Hyalomma aegyptium*, although among batches of infected ticks, never showed infection.

   (*c*) The cows from which the infected ticks were collected were invariably healthy.

2. *The infection was not contaminative*, because:—

   (*a*) Ticks, unlike other blood-sucking parasites generally, do not move about much on their hosts (except the males), but settle in one place and slowly secure an attachment, where they remain till replete.

   (*b*) The natural secretions and excretions dry very rapidly, and are often voided on the earth after the tick detaches itself from its host.

3. *The infection was hereditary*, because:—

   (*a*) The height of crithidial infection occurred about the time of oviposition.

   (*b*) The occurrence of the infection in batches of ticks all of which probably belong to the same generation.

The practical application of these lines of thought worked out as follows:—

1. The intestinal diverticula of the ticks were never found to harbour parasites.
2. The haemocoelic fluid was the habitat of the early pre-flagellate forms.
3. These early pre-flagellate forms developed into full grown Crithidia in the haemocoelic fluid. When these were present in large numbers they were found to migrate to the ovarian system.
4. The hereditary infecting forms were found entering the ova.
5. Plasmodial forms were found in the deposited eggs.

MATERIAL AND METHODS

Many specimens of *Hyalomma aegyptium* were examined from different localities, but always from cows, which are their common hosts. Some batches of ticks never showed infection, while in others infection was the rule.

To study the living organism, living specimens were examined with ordinary light and with dark ground illumination, but with the latter the Crithidia were found to degenerate and die rapidly because of the strong light and heat produced by the source of illumination used in working with a paraboloid condenser. Vital staining was also employed.

To obtain the haemocoelic fluid a portion of any of the legs is removed with a fine pair of scissors. For stained specimens of tissues all ticks were carefully dissected under a dissecting microscope, and the organs after carefully washing in saline solution, as recommended by Sir William Leishman, were smeared out on slides.

Osmic acid and alcohol were employed as fixatives, and also special methods of wet fixation were used, such as Schaudinn's fluid (corrosive-acetic-alcohol), and Bouin's fluid (picro-formol-acetic-alcohol).

Various stains were used—Leishman's and Giemsa's solutions gave good results. Bohmer's haematoxylin (which in this climate is rather superior to Delafield's haematoxylin) and Heidenhain's iron haematoxylin and its modifications were employed.

Cultivation of the flagellate has proved negative up to the present, together with attempts to inoculate animals. Frequent examinations and cultivation of the blood of the cows have given negative results.

DISTRIBUTION OF THE PARASITE IN THE HOST

The majority of insect flagellates are found parasitic in the alimentary tracts of their hosts, but in this respect the Crithidia of *Hyalomma aegyptium* is an exception. The haemocoelic fluid is the seat of election for the development and subsequent distribution of the parasite in the adult tick.

Shortly after removal of the ticks from their hosts (cattle in this instance) the early pre-flagellate forms may be found in the haemocoelic fluid (Pl. XXXVIII, figs. 1-5), which is a dark amber colour, and is of about the consistency of human blood serum. Microscopically it is seen to contain among other elements the large vacuolated haemocoelic corpuscles. The nuclear and protoplasmic portions of these cells, when coloured with Leishman's or Giemsa's

solutions, show a tendency to stain, as do the nuclei, blepharoplasts and protoplasm of human and animal parasites. This point is of importance, as fragments of these cells might easily be mistaken at first sight for parasites.

A few days after the ticks have been collected, the early pre-flagellate forms show evidences of division and increase in number (figs. 5, 6, 8).

After about four to ten days, that is, a little time previous to oviposition, early flagellate forms make their appearance (figs. 11-18). Rapid multiplication of the young flagellates now takes place, with the formation of division rosettes (figs. 18 and 19), and the growth to adult Crithidia (Pl. XXXVIII, figs. 20-29; Pl. XXXIX, figs. 31-33) soon follows:—

The fully-grown Crithidia multiply by longitudinal division (Pl. XXXIX, figs. 30, 34-39) till the smallest drop of haemocoelic fluid presents a felted mass of crithidial bodies and waving flagella. Owing to the great numbers of parasites present, the haemocoelic fluid is a milk-white colour. Towards the end of oviposition, and just previous to the death of the tick, post-flagellate forms may be found (figs. 40-44) in the haemocoelic fluid, although in some cases all forms disappear from it. The post-flagellate forms also occur in the genital tract, and are perhaps better termed ovarian forms: they will be dealt with under the description of hereditary infection.

The intestinal diverticula and malpighian tubes have not been found to harbour any of the parasites in the adult tick. Sometimes early division forms (Pl. XL, fig. 62) are found in the salivary glands, but this seems to be the exception rather than the rule.

The parasites were found more commonly in female than in male ticks.

MORPHOLOGY

The life cycle of *Crithidia hyalommae* in the adult tick may, for the sake of description, be divided into four periods which, however, merge into each other. These are the pre-flagellate, the flagellate, the post-flagellate, and ovarian stages. The morphology of the ovarian stage will be dealt with under a subsequent heading. The morphology of the pre-flagellate, the flagellate and post-flagellate stages may be described as follows:—

The Pre-flagellate Stage

The early pre-flagellate stages of *C. hyalommae* found in the haemocoelic fluid are rounded or oval Leishmania-like bodies (Pl. XXXVIII, figs. 1-5, 7, 8), which vary from 4 to 12μ in length and from 4 to 11μ in breadth. The protoplasm of these forms is of a finely reticulate nature, containing distinct vacuoles (fig. 4) and a rounded nucleus which is situate near the periphery of the parasite (figs. 1, 2). The blepharoplast is rounded or bow shaped (figs. 1, 2), and acquires the characteristic dark tint of such structures when coloured by the various stains. It may be placed at the side of, or opposite to, the nucleus (figs. 3, 4).

These early forms soon show evidences of division (fig. 5), two daughter parasites resulting, smaller in size and with more granular protoplasm than their precursors. Plasmodial forms (fig. 6) may result from rapid division. Increase in the size of the flagellate bodies follows and the flagellum makes its appearance, arising in the neighbourhood of the blepharoplast. The flagellum in its outward growth carries with it a portion of the periplast which forms the undulating membrane.

After the formation of the flagellum rapid division takes place, rosettes being formed (figs. 18, 19), indeed nearly all stages from rounded flagellates to nearly adult Crithidia may be seen in the same specimen and in the same rosette (figs. 18, 19, 27). It may also be noted that individual members of a division rosette grow at unequal rates. The aflagellar end now grows outwards, at first wide and blunted (figs. 25, 26, 29), but it soon narrows down and tapers off into a fine point (figs. 27, 28).

The Flagellate Stage

The fully-grown flagellates present the crithidial type of body, but are unusually long and slender (Pl. XXXIX, figs. 28a-36). The length of the body and flagellum varies from 26μ to 50μ, the length of the flagellum alone being 12·5 μ. The average breadth of the nucleus varies from 2 μ to 2·5 μ.

The aflagellar extremity tapers gradually to a fine point, while the flagellate end of the body is continued for some distance along the flagellum.

The flagellum, taking its origin from the vicinity of the blepharoplast, runs along the free border of the undulating membrane until the extremity of the body is reached. Here it escapes from the periplast and projects freely for a considerable distance. The flagellum is never connected with the blepharoplast. A basal granule has been noted (fig. 31), but its presence is not always evident, probably because of its close relation to the blepharoplast. The undulating membrane is well marked, and in living specimens its movements may be observed. In stained preparations it may be seen thrown into folds.

The nucleus is roundish (figs. 28a-36) or oval, and generally lies about the middle of the body, the whole breadth of which it nearly occupies (figs. 28a-36). The structure of the nucleus varies. At times the nucleus appears somewhat vesicular with a marked karyosome (figs. 31-33); at other times the chromatin of the nucleus is distributed in granules within the nuclear membrane (fig. 30). When division is about to take place, the nuclear material is generally more granular (fig. 30).

The blepharoplast of *C. hyalommae* is well defined in all stained specimens, and colours deeply. Its position varies within what might be termed the limits of crithidial definition (Patton, 1908). It may be closely applied to or a little distance from the flagellar or aflagellar side of the nucleus (figs. 28a-33).

In all cases the flagellum arises in the close vicinity of the blepharoplast, in whatever position the latter may be situated. Two blepharoplasts may often be observed (figs. 30 and 35-38), but this is really an early division phase. The blepharoplast is usually rod-shaped (fig. 28a), and sometimes shows a diplosome appearance (fig. 34), probably indicative of approaching division.

Chromatoid granules may be present in the protoplasm. In fresh specimens the protoplasm of the flagellar extremity of the body is markedly granular.

The Post-Flagellate Stage

The post-flagellate forms seen in the haemocoelic fluid are merely rounding-up parasites (Pl. XXXIX, figs. 40-44). They are similar to the ovarian forms which undergo the same process in

the walls of the female generative tract, and which will be described when dealing with the hereditary infection.

Division

In the pre-flagellate stages division is initiated by the blepharoplast, which divides into two nearly equal masses. These masses now move one on either side of the nucleus (Pl. XXXVIII, fig. 5), or form the base of a triangle with the nucleus at the apex (fig. 8). The nucleus now divides into two, and when the binucleate system is completed the protoplasm splits, two daughter forms resulting. Large plasmodial forms may be produced in the haemocoelic fluid before the flagella are formed (fig. 6).

In the early flagellate stages, the same phenomena are observed; the blepharoplast is often bow-shaped before splitting (fig. 11). The flagellum divides, commencing at its blepharoplastic end (fig. 11) with the division of the blepharoplast and basal granule. The blepharoplast and flagellum may have divided, and protoplasmic cleavage may have commenced before the nucleus has completed its division (fig. 15). The lashing movements of the flagella at this stage may assist in this early protoplasmic division. Fig. 15 shows this condition, with details of nuclear division: the nucleus still shows the mantle fibres surrounding the centrodesmose which connects the equatorial plates.

Division in the early flagellates is rapid, and division rosettes are formed (figs. 18, 19). Two or more phases in division may be observed in the same rosette (figs. 18, 19).

In the full-grown Crithidia longitudinal division takes place. Before division the blepharoplast presents a diplosome appearance (Pl. XXXIX, fig. 34), and divides first, with the flagellum (fig. 35). One portion of the blepharoplast and its future flagellum migrates to the opposite side of the nucleus. The blepharoplasts may be connected together by a thin chromatin line (fig. 36).

The nucleus, which is generally granular at this stage, divides into two. The two flagellar ends separate, and the flagella, moving rapidly, separate the remainder of the organism into two similar adult Crithidia.

Division of the post-flagellate stages may occur in the haemo-

coelic fluid, but takes place to a marked extent in the ovarian tissues (Pl. XXXIX, figs. 51, 52), ovarian cells (Pl. XL, figs. 55-57), and young ova (Pl. XL, figs. 60, 61). Their division is similar to the division of the pre-flagellate stages, and requires no special description. Aggregation rosettes are formed (Pl. XXXIX, fig. 50).

Multiple division takes place in the deposited ova as in the early stages in the haemocoelic fluid. These forms, the result of multiple division, are multi-nucleated masses of protoplasm, and have been termed 'plasmodial forms' (Pl. XL, figs. 60, 61).

THE HEREDITARY INFECTION

The proofs of the occurrence of hereditary infection may be studied by the examination of living specimens and stained preparations. The process of events in the ovarian system is as follows:

About the time of oviposition, and during this event, the adult Crithidia pass by means of the haemocoelic fluid to the ovary and oviducts.

A large number of the crithidial forms here begin the post-flagellate ovarian stage of their existence in the walls (Pl. XXXIX, figs. 45-54) and cells (Pl. XL, figs. 55-57) of these organs. This post-flagellate or ovarian stage begins with the absorption of the flagella and the gradual rounding off of the parasites (Pl. XXXIX, figs. 45, 46) to Leishmania-like forms (figs. 47-49). A very large number of the parasites perform this evolution (fig. 50). A considerable number of the adult Crithidia, however, pierce the walls of the oviducts, preferably high up where the oval capsules are soft. Here they proceed to pierce the walls of the ova (Pl. XL, fig. 58), more than one parasite often entering the same ovum (Pl. XL, fig. 59). The aflagellar end is the foremost end in entering. A boring motion may now be observed, the flagellum describing a propeller-like movement, the whole organism being screwed forward, the undulating membrane forming the thread of the screw. Another motion has also been observed in the ovarian forms. After rounding of the protoplasm of the flagellar end, a circular motion has been noted which occurs previous to the disappearance of the flagellum and formation of the resting

bodies. Once within the egg the rounding-up process described above takes place, and the Leishmania-like stage results. Division of these small forms may be found in the ova (Pl. XL, figs. 60, 61), which ultimately break up into small resting bodies. A considerable number of the ova of infected ticks dry up and die. Generally the later deposited ova contain the resting forms; the ova deposited early in oviposition are usually non-infected.

SUMMARY

1. *Crithidia hyalommae* is a flagellate parasite occurring in the haemocoelic fluid, salivary glands, ovary, oviducts and ova of *Hyalomma aegyptium*, the common cattle tick of the Anglo-Egyptian Sudan.

2. The parasite has four periods in its life-cycle: a pre-flagellate stage (Pl. XXXVIII, figs. 1-8) passed chiefly in the haemocoelic fluid, a flagellate stage in the haemocoelic fluid (Pls. XXXVIII, XXXIX, figs. 9-39) and in the ovary and oviducts (Pl. XXXIX, fig. 50 and Pl. XL, figs. 58, 59), a post-flagellate stage (Pl. XXXIX, figs. 40-44) in the haemocoelic fluid, and a post-flagellate or ovarian stage in the ovary and oviducts (Pl. XXXVIII, XXXIX, figs. 45-54), ovarian cells (Pl. XL, figs. 55-57), and ova (Pl. XL, figs. 60, 61).

3. The pre-flagellate stage is of short duration. The parasites are small rounded or oval bodies from 4 μ to 11 μ broad, and from 4 μ to 12 μ long. Their protoplasm is reticulate or granular, depending on division. They contain a nucleus and a rod-shaped blepharoplast. Division of the very earliest forms takes place (Pl. XXXVIII, figs. 5-8).

4. The flagellate forms vary from 26 μ to 50 μ in length and from 2 μ to 2·5 μ in breadth.

The protoplasm is reticulate. The consistency of the nucleus varies with division. The rod (Pl. XXXIX, fig. 28a) or diplosomic blepharoplast (fig. 34) is usually situated on the flagellar side of the nucleus, but its position varies at times, depending on the phase of division. The flagellum arises in the neighbourhood of the blepharoplast, but not from it.

5. The undulating membrane and flagellum are both well marked. The undulating membrane assists considerably in motion.

The flagellum forms the border of the undulating membrane, and continues free from the protoplasmic body for a considerable distance.

A basal granule has been noted (Pl. XXXIX, fig. 31).

6. The post-flagellate and ovarian stages occur in the haemocoelic fluid, ovary and oviducts. A superfluity of the ovarian forms seems to be present, a large number of them entering a resting stage in the ovarian cells and tissues.

7. The ovarian stages of *Crithidia hyalommae* serve for the hereditary transmission of the parasite. The flagellates pass by means of the haemocoelic fluid to the ovaries and oviducts; here they pierce the ova, the aflagellar end of the parasite being anterior. Within the ova the flagellates round up and undergo subsequent division with the production of 'plasmodial' forms.

8. Multiplication of *C. hyalommae* by longitudinal division takes place in all its stages.

9. Infection of *Hyalommae aegyptium* by *Crithidia hyalommae* in the adult is purely a hereditary infection.

10. The flagellate is a strictly parasitic flagellate of the tick, and is non-pathogenic to the tick host.

Acknowledgments

I wish to thank Dr. Balfour, the recent Director of these Laboratories, and Dr. Chalmers, the present Director, for their valuable assistance in both work and text; also Dr. Fantham, whose experience in this line of research is well known, for his valuable advice in the text and aid with the drawings. To Dr. Bousfield and his able staff I am indebted for a plentiful supply of working material.

Khartoum,

September 27th, 1913.

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EXPLANATION OF PLATES

PLATE XXXVIII

All figures were outlined with the Abbé-Zeiss camera lucida, after magnification with a 2 mm. apochromatic objective (Zeiss) with No. 12 compensating ocular.

The magnification of the figures is approximately 2,000 diameters.

Figs. 1-4. Early pre-flagellate forms of *Crithidia hyalommae* from the haemocoelic fluid.

Fig. 5. Young dividing form from haemocoelic fluid.

Fig. 6. Plasmodial form from haemocoelic fluid.

Figs. 7-8. Young forms from haemocoelic fluid.

Figs. 9-10. Rounded form showing flagellum.

Figs. 11-12. Early flagellates in haemocoelic fluid.

Figs. 13-16. Division phases in early flagellates.

Fig. 17. Early flagellate in haemocoelic fluid.

Figs. 18-19. Division rosettes, haemocoelic fluid.

Figs. 20-29. Development to full grown Crithidia, haemocoelic fluid.

Plate XXXIX

Fig. 28a. Fully grown Crithidia from haemocoelic fluid showing rod-shaped blepharoplast.

Fig. 29a. Fully grown flagellate from haemocoelic fluid showing blepharoplast on flagellar side of nucleus.

Figs. 31-33. Showing different positions of blepharoplast.

Figs. 34-36. Commencing division of blepharoplast. Fig. 34 shows diplosome blepharoplast. Fig. 36. Parasite showing nuclear membrane and chromatic band from haemocoelic fluid.

Figs. 30, 37, 38, 39. Stages in longitudinal division from haemocoelic fluid.

Figs. 40-44. Post-flagellate stages in haemocoelic fluid.

Figs. 45-49. Post-flagellate or ovarian stage in the ovary and oviducts.

Fig. 50. Shows aggregation rosette of ovarian stages in walls of oviduct.

Figs. 51-52. Division stages of Leishmania-like forms of ovary.

Plate XL

Figs. 53-54. Division stages of ovarian forms of *Crithidia hyalommae.*

Figs. 55-57. Resting and division stages in ovarian cells.

Fig. 58. Entry of adult Crithidia into wall of oviduct.

Fig. 59. Perforation of egg by adult Crithidia.

Figs. 60-61. Plasmodial forms in deposited ova.

Fig. 62. Early form (rare) in salivary gland.

TRYPANOSOMA VIVAX IN RABBITS

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(*Received for publication* 6 *October*, 1913)

It is generally accepted that *T. vivax* (*T. cazalboui*) is not pathogenic for small laboratory animals. Thus Laveran and Mesnil,* writing on *T. cazalboui*, state that the dog, monkey, rabbit, guinea-pig, rat and mouse are refractory, and that this fact constitutes one of the chief characters of the trypanosome. It is true that Ziemann† records infection with *T. vivax* in dogs and rats, but as short aflagellar trypanosomes were found by Ziemann in his sheep and goats, it is highly probable that the animals from which the dogs and rats were inoculated had a double infection of *T. vivax* and *T. congolense*, and that the sub-inoculated animals became infected with the latter parasite only. We might remark in this connection that of late years it has been shown that double infection of domestic animals and game with these trypanosomes is by no means uncommon.

In previous communications‡ we have recorded an instance of such a double infection in a horse naturally infected in the Gambia, and described the manner in which the two parasites were isolated one from the other.

This horse was the source of the strain of *T. vivax* with which the present paper deals. As mentioned in a former paper,§ the *vivax* strain was separated fortuitously from the short aflagellar parasite by inoculation of goats from the horse at different dates; one of the sub-inoculated goats developed a pure culture of

* Laveran & Mesnil. Trypanosomes et Trypanosomiases. Paris, 1912, p. 542.

† Ziemann. Beitrag zur Trypanosomen Frage. Centralb. f. Bakt. Orig., 1905, Bd. XXXVIII, p. 307, 429-662.

‡ Yorke & Blacklock. The trypanosomes found in two horses naturally infected in the Gambia. Annals of Trop. Med. & Parasit., Vol. V, No. 3, p. 413.

§ Blacklock. The trypanosomes found in a horse naturally infected in the Gambia. A double infection. Annals of Trop. Med. & Parasitology, 1912, Vol. VI, No. 1B, p. 107.

T. vivax. From this time (June 14, 1911) up to the present the strain has been preserved by passage through a series of thirty-nine goats.

It is of interest to note that in spite of direct inoculation of the strain from goat to goat during a period of nearly two and a half years, no appreciable increase of virulence has been observed. Thus the average duration of the disease in the first fourteen animals used was thirty-one days, whilst that in the last fourteen was twenty-nine days. The periods of incubation and duration of the disease in this series of goats are given in Table I.

TABLE I. Showing a series of thirty-nine passages of *T. vivax* in goats during a period of twenty-eight months.

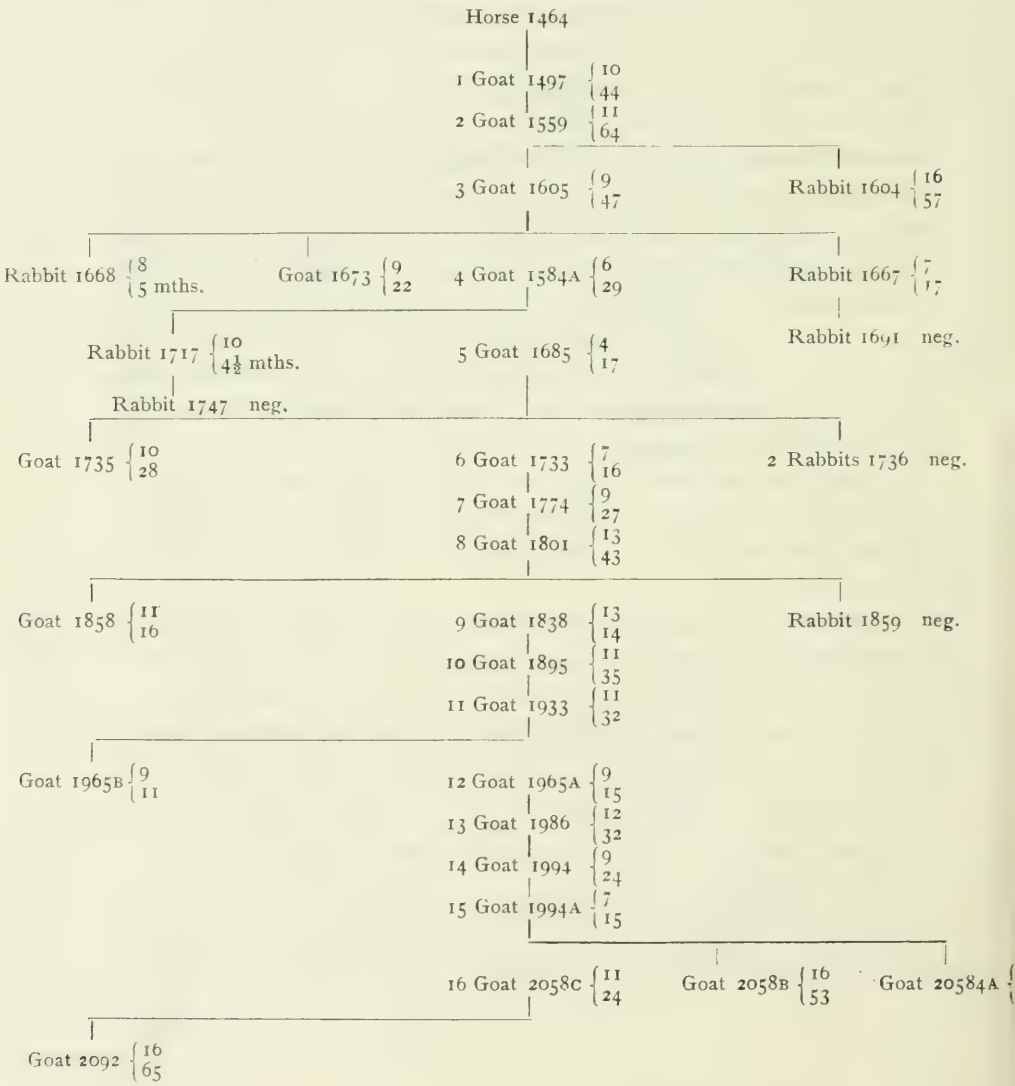
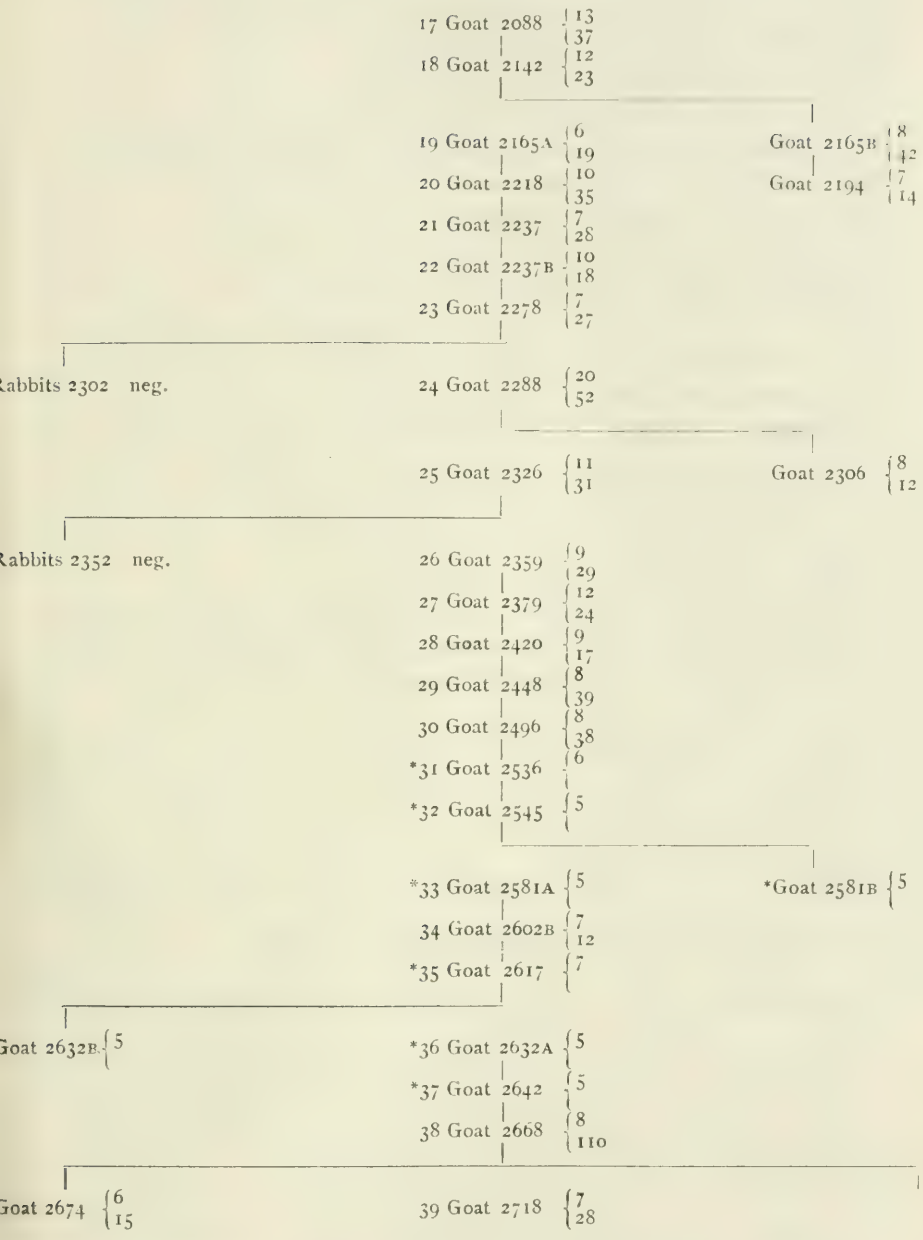


TABLE I—*continued*



* Goats marked thus used for treatment.

The upper figures in each bracket indicate incubation; the lower, duration.

Attempts to infect rabbits, made in the earlier passages of the strain through goats, either failed entirely or resulted in a temporary infection in which the parasites were exceedingly scarce and disappeared after a few days.* It was not possible to carry on the strain to a second generation in rabbits.

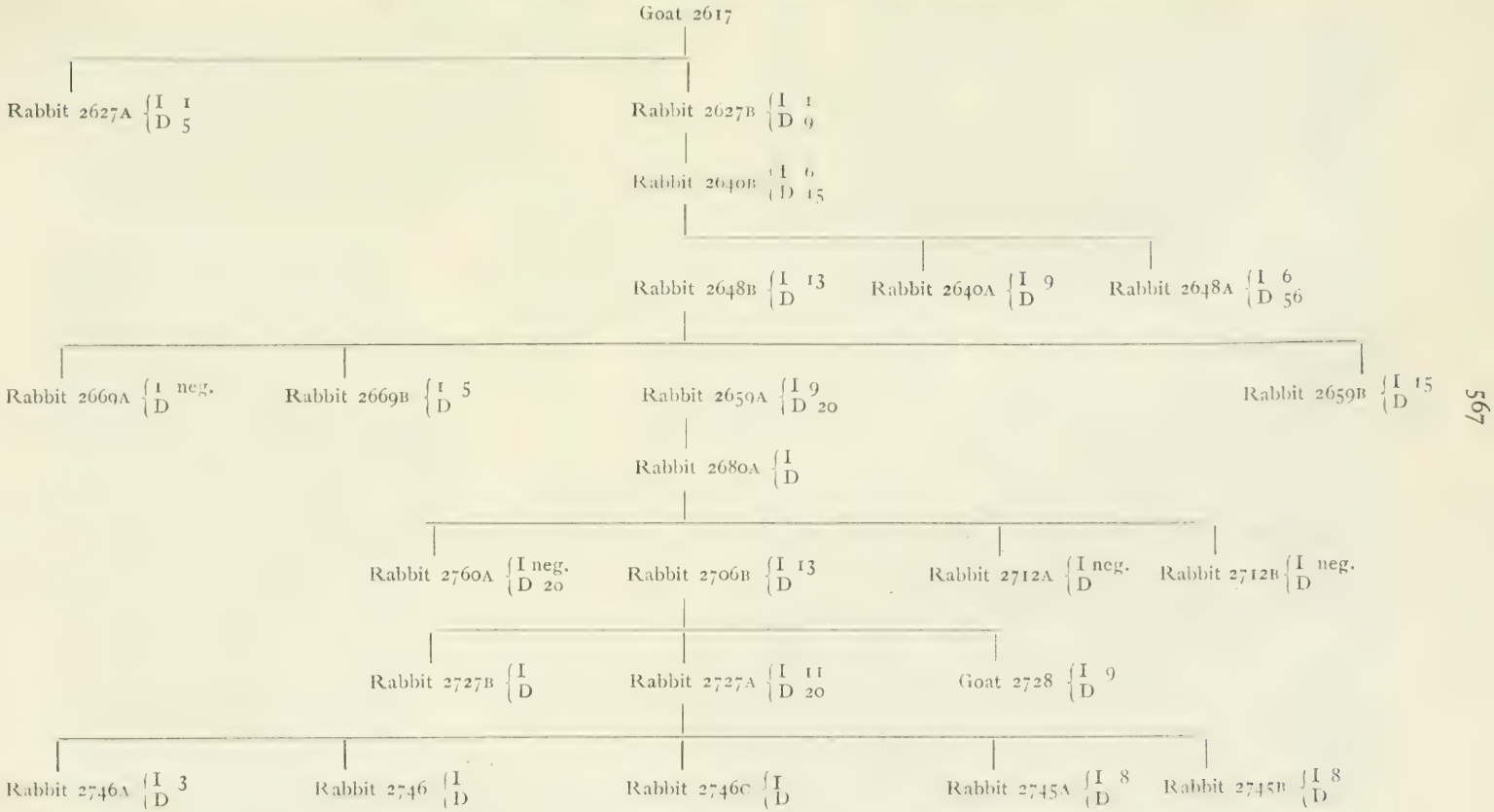
Inoculation made from the thirty-eighth goat proved, however, to be more successful, and four rabbits, injected intraperitoneally with small amounts (0·5 to 1 c.c.) of the goat's blood, all became infected after incubation periods of five to eight days. The infection in all was well marked, and parasites were fairly numerous in the peripheral blood—up to twenty to a microscope field. Two of the four died on the seventeenth and thirty-fourth days, respectively, with numerous parasites in the peripheral blood. The spleen was greatly enlarged and the animals were very emaciated; the weights had decreased from 1220 to 970 grams and from 1390 to 1000 grams. There has been some loss of weight in the other animals, but after exhibiting parasites in the peripheral blood for from three to eleven days they have since become negative, and appear to have recovered.

There is, hence, evidence that the strain is, after thirty-eight passages through goats, to a certain extent virulent for rabbits. Three rabbits were inoculated intraperitoneally from the thirty-fifth goat with about 30 c.c. of defibrinated blood. Trypanosomes were found in the peripheral blood on the next day, and remained in considerable numbers—as many as fifty to a microscope field—until the time of death, which occurred on the fifth, ninth and fourteenth days, respectively. All the animals presented marked signs of infection, the spleens were greatly enlarged, and the animals had lost a considerable amount of weight.

From one of these (Rabbit 2627B), two others were inoculated intraperitoneally with 1 c.c. of citrated blood (containing about 0·25 c.c. of blood). Trypanosomes were found in the peripheral blood of one of the two sub-inoculated animals on the sixth, and it died on the fifteenth day after inoculation. The strain has since been carried on in rabbits over a period of more than three months, until the eighth generation, as shown in Table 2.

* Blacklock. A note on *T. vivax* in rabbits and white rats. Annals of Trop. Med. & Parasitology, 1912, Vol. V, No. 4, p. 537.

TABLE II.—Showing a series of eight passages of *T. vivax* in Rabbits during a period of three months.



The upper figure in each bracket indicates incubation; the lower, duration.

From a rabbit of the sixth generation a goat was inoculated subcutaneously with 1 c.c. of citrated blood, and trypanosomes appeared in its blood on the ninth day.

Although, as the table indicates, the strain has not yet become invariably pathogenic for rabbits, and a considerable number appear to have recovered, nevertheless most of the animals have developed a definite infection, and in five the disease has run an exceedingly acute course, the animals dying in from five to twenty days with numerous parasites in the peripheral blood.

We have examined the strain after passage through six generations of rabbits, with a view to ascertaining whether it has undergone morphological changes. In this connection we might mention that as a general rule the trypanosomes in rabbits do not exhibit that rapidity of movement which is characteristic of them in goat's blood. In stained preparations the trypanosomes appeared to be identical with those seen in goat's blood.

We intend, if possible, to carry on this strain in rabbits in the hope of rendering it acutely pathogenic for other small laboratory animals.

THE PATHOGENICITY OF *NOSEMA APIS* TO INSECTS OTHER THAN HIVE BEES

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CONTENTS

		PAGE
I.	INTRODUCTION	569
II.	EXPERIMENTAL INFECTION OF VARIOUS INSECTS	570
	A. HYMENOPTERA	
	(i) Humble Bees	570
	(ii) Mason Bees	570
	(iii) Wasps	571
	B. LEPIDOPTERA	
	(i) Cabbage White Butterflies	572
	(ii) Peacock Butterflies	572
	(iii) Cinnabar Moths	573
	(iv) Gooseberry Moths	574
	C. DIPTERA	
	(i) Blow Flies	575
	(ii) Crane Flies	576
	(iii) Sheep Keds	576
III.	SUMMARY AND CONCLUSIONS	578
	REFERENCES	579

I. INTRODUCTION

Nosema apis is now well known as the minute, microsporidian parasite that causes a malady of hive bees, popularly known as 'Isle of Wight' disease, though it should be noted that several diseases of bees are often being confused under this general name. The organism, *N. apis*, is transferred from bee to bee by means of resistant spores in the dejecta of the infected host, which spores are taken up by other bees, either with food or drink, or during the various processes consequent on the communal life of the bees.

The Nosema spores absorbed by the bee liberate amoeboid forms termed planonts, which enter the epithelial cells of the gut and

develop rapidly, particularly in the chyle stomach of the bee where they undergo rapid, asexual multiplication, forming meronts. The functional derangements resulting therefrom are quite sufficient to bring about the death of the host in many cases. Should the host react successfully on the parasites, spore formation ensues, and the Nosema leave the host as highly resistant spores that are well adapted for extra-corporeal life, and that serve to perpetuate the species should they be able to reach a new host.

Some experiments have been carried out with insects other than *Apis mellifica*, and tend to show that the pathogenicity of *Nosema apis* is far from restricted to the hive bee. Many of the experiments were suggested by observation of the habits of various insects present when examination of bees was in progress. Although the numbers of the insects used were not as large as we could have wished, the results are of interest and importance.

II. EXPERIMENTAL INFECTION OF VARIOUS INSECTS

Experimental infections of members of the Hymenoptera, Lepidoptera and Diptera have been made.

A. *HYMENOPTERA.*

The experimental insects used were humble bees, mason bees and wasps.

(i) HUMBLE BEES (*Bombus terrestris*, *B. lapidarius*, *B. hortorum*, *B. venustus*, *B. latreillelus*). It has been shown previously by Dr. Graham Smith and ourselves that a Nosema is a natural parasite of humble bees (*Bombus spp.*). It seems to be a different species of Nosema from that so destructive to hive bees, and chiefly parasitises the malpighian tubules of the humble bees.

But humble bees belonging to apparently clean stocks, when kept in captivity and provided with food contaminated with excrement of hive bees containing *Nosema apis* spores, both can, and do, become infected with the parasite, and die from the effects thereof. Both meronts and spores can be found in the walls of the gut, while the malpighian tubules, as is usual with infections of *Nosema apis*, remain uninfected. Humble bees used as controls and supplied with pure food showed no Nosema.

(ii) MASON BEES. The colonies of mason bees used were

brought from abroad and were lodged in a piece of old wall. Some of the bees were isolated to act as controls. The rest were kept in a wall, screened off to prevent exit, and were supplied with honey and pollen contaminated with spores of *Nosema apis* from dead bees. The abundant food supply proved attractive and the bees fed somewhat greedily. On the fourth day after the first supply of contaminated food, it was noted that fewer bees flew out, and a few dead ones were found. On dissection, the alimentary tract of these bees was shown to be parasitised with meronts of *N. apis*. The dwindling of the bees continued, and some were found unable to fly. *N. apis* in the form of meronts and young spores was demonstrable on examination. Ultimately the two colonies supplied with *N. apis* died out, and the brood also was found dead. A few larvae showed Nosema spores, but the brood had been too long dead when recovered for the determination of young stages of the parasite. When both colonies were extinct, the controls that had been supplied with pure honey were examined. Seven-eighths of them had survived, and when these were dissected no parasites were found. The controls that died were also dissected, but as their food canals were empty, and their fat bodies greatly reduced, their deaths were ascribed to voluntary starvation.

(iii) WASPS (*Vespa germanica*). Two years ago we reported that a colony of wasps had been exterminated by introducing bees dead of Nosema into their nest. Since then, the experiment has been repeated, with similar results, and examination of recently dead wasps has shown that multiplicative stages (meronts) of *Nosema apis* were present in numbers in the alimentary tracts of the wasps.

Again, wasps collect dead and dying bees and carry them away as food for their larvae. Several cases are known to us in which the wasp broods have died out entirely as the result of a liberal diet of bees dead of Nosema. As before, the parasite underwent development in the body of its host, and in the majority of the wasps, adults and larvae alike, the host was killed before the life-cycle of the parasite was completed by spore formation. The hypothesis that the newer a parasite is to its host, the greater is its virulence, thus receives further support.

B. *LEPIDOPTERA.*

Certain common Lepidoptera were used for experimental purposes, controls being kept in each case.

(i) CABBAGE WHITE BUTTERFLIES (*Pieris brassicae*). Both larvae and imagines were used for experiment, as both could possibly acquire *Nosema apis* naturally with their food.

(*a*) An adult, ♂, was fed on the day it emerged from the pupa on syrup made with castor sugar and contaminated with spores from the gut of a bee. The butterfly was fed again three days later, and died on the fifth day after the first feed. Nosema spores and young stages of the parasite were found in the gut. The control died on the sixth day, and no parasites occurred in it.

(*b*) An adult, ♀, was fed on the second day after escape from the pupa on sugar syrup contaminated with spores. It refused to feed on the first day. It lived four days after emergence. A few Nosema meronts were found in the walls of the gut after death. A control lived six days.

(*c*) Larvae. Cabbage plants near a badly diseased hive were found spattered with bee excrement. Dead larvae of cabbage white butterflies also were found. Several experiments were then made, of which the one cited was typical. Eight larvae were collected from clean cabbage plants. Four were fed on a cabbage leaf smeared with honey in which an infected bee's gut had been emulsified, the remaining four on ordinary clean cabbage leaves.

One experimental larva died after two days with Nosema infection in its gut. Two, also containing Nosema, died on the following day. The remaining one pupated on the third day, but the imago never emerged. Of the four control larvae, all pupated and produced imagines in due course.

(ii) PEACOCK BUTTERFLIES (*Vanessa io*). On two occasions when bees were under examination for *Nosema apis*, peacock butterflies came into the laboratory, and after the usual aimless flutterings, settled on the viscera of dead bees and proceeded to suck honey from the contents. Both were captured and were examined after death. A second butterfly was captured on each occasion to act as control.

(*a*) Adult, ♂. It lived two days after capture. When

dissected, no stages of *Nosema apis* were found in the gut walls, and only a very few Nosema spores—probably those ingested with the food—were present.

(*b*) Adult, ♀. This was a rather large female. Soon after feeding, oviposition began, and the insect died at its termination. No Nosema was found in it.

The extremely small number of experiments prevents any definite conclusion being reached as to the pathogenicity of the protozoon to the insect. All that can be said is that a negative result was obtained in the two cases investigated.

(iii) CINNABAR MOTHS (*Callimorpha jacobeae*). Larvae of the cinnabar moths were used for experiment as they were observed on several occasions feeding on groundsel contaminated with bee excrement. The subjects experimented on were obtained from a locality where bee-keeping was not practised, and where the larvae seemed remarkably healthy.

Fifteen young caterpillars of the cinnabar moth were placed on a plant of groundsel growing in a flowerpot and covered by a bell jar to prevent escapes. The groundsel was watered with an emulsion of dead bees containing Nosema spores, filtered through coarse muslin that readily allowed the Nosema spores to pass through, but retarded the passage of the chitinous portions of the bees. The groundsel foliage was watered daily. The larvae fed on the groundsel in a quite normal way. Control larvae were kept under similar conditions but their groundsel was watered with ordinary tap water. These control larvae never showed any form of *Nosema apis*. The results of the experiment were:—

Three caterpillars, fed on infected groundsel, died two days after the first infective feed. Young stages of *Nosema apis* were present in their mid guts. The hind gut was not affected. The parasites were in the multiplicative phase, meront formation being in progress.

Seven larvae died on the fourth day. Of these, one contained young spores of *Nosema apis*, two had a very heavy infection of young meronts in an actively dividing condition, and the remaining four showed a few young stages of the parasite. The condition of the guts of the larvae resembled that seen in bees, and the varying degrees of infection among the larvae are parallel to what

we have so often found when examining a series of bees from one colony.

One larva died on the fifth day after the first feed, and contained meronts of *Nosema apis*. On the same day one pupated, and ultimately a deformed male imago issued from it. This insect lived three hours only. It was dissected as soon as possible after death, but no stages of *Nosema apis* could be recognised with certainty within it.

The remaining larvae died during the night and were too decomposed when examined to permit the detection of the planonts or meronts of *Nosema apis*, had they been present. No spores were found.

The life cycle of *Nosema apis* as seen in the larvae of the cinnabar moth was the same as that found in the hive bee. The spores gave rise to planonts, which became meronts in the epithelial lining of the alimentary tract, and these, in turn, produced spores.

(iv) GOOSEBERRY MOTHS (*Abraxas grossulariata*). Observations on a garden in a district heavily infected with apian microsporidiosis showed that, in one case, the foliage of gooseberry bushes near infected hives was spattered with the excrement of the bees, and the dejecta on the leaves contained spores of *Nosema apis*. Beneath the bushes a few dead larvae of the gooseberry moth were found, and as the body of one of these contained Nosema spores, experiments with larvae obtained from an uninfected district were commenced. Twelve larvae were divided into two sets of six. Both were fed on gooseberry twigs, the twigs being moistened daily with equal quantities of water, and of water containing Nosema spores, respectively. The results may be summarised thus:—

(*a*) *Larvae supplied with food contaminated by Nosema spores.* On the fifth day after the first infective feed, one larva died. Nosema spores were found in its intestine, toegther with meronts. These spores were fed to bees and reproduced the disease.

On the seventh day two more larvae were found dead. Thirty-six hours had elapsed since they were last seen alive. Decay had been rapid, and it was impossible to identify young stages of *Nosema apis*, and no spores were found. Thus it is uncertain whether these two larvae became infected.

One larva died on the eighth day. It contained both spores and meronts of *Nosema apis*.

Two larvae pupated on the tenth day. The pupae were small compared with those from the control larvae. One pupa was dissected on the twelfth day, and a few meronts were found in the body. The second pupa did not produce an imago. Whether this was the effect of the action of the Nosema cannot be stated with certainty.

(*b*) *Larvae feeding on uncontaminated food.* Two of the larvae were dissected. Neither showed any trace of *Nosema apis*. The remaining four pupated. Two pupae were dissected and examined. *Nosema apis* was absent, nor was any other parasite found in them. Of the remaining two pupae, one produced a beautifully shaped and marked female, but the second did not develop.

Morphologically, the Nosema present in the larvae of the gooseberry moth differed in no wise from the parasite as seen in bees, and its identity was established by feeding bees with the spores obtained from the caterpillars and thereby reproducing the disease.

C. DIPTERA.

The Diptera used for experiment were blow flies, crane flies and sheep keds.

(i) BLOW FLIES (*Calliphora erythrocephala*). During the examinations of bees for *Nosema apis*, it was noticed that blow-flies settled on the viscera of the bees and fed upon them. The flies were also often seen sucking up the sweet excrement voided on the alighting board and sides of the hive; they subsequently showed Nosema infection.

A number of pupae of *Calliphora erythrocephala* were dug up in a garden on March 26th, 1912. The adult flies began to emerge on March 28th, but could not be induced to feed on that day. On March 29th, the flies sucked a piece of meat with some Nosema spores on it. The following day they were supplied with moistened sugar contaminated with Nosema spores. They refused again to feed, but sat on the sugar most of the time.

From April 1st to April 9th, the blow flies hatched out at the

rate of one or two a day, and were formed into experimental and control sets. Special cases are now cited:—

Blow Fly 1 emerged from the pupa. It was provided with contaminated sugar and sucked it readily. It died nine days after emergence, and on dissection showed some young stages and a very few spores of *Nosema apis* in its gut. Infection of the Malpighian tubules also occurred.

Blow Fly 2 was smaller than Fly 1, and emerged a day later than it. It also lived nine days, while its control lived ten days. At death it contained a number of Nosema spores.

Blow Flies 3, 4, 5 and 6 lived from 7 to 10 days after emergence. All were fed on infected candy. Blow Fly 3 became infected, showing a few spores at death. Blow Fly 4 showed no form of *Nosema apis*. Blow Flies 5 and 6 contained a very few spores of *N. apis*. Blow Fly 7 showed no *Nosema apis*, but contained what is probably a new species of Nosema. Blow Fly 8 contained many young stages of *Nosema apis*, together with a fair number of spores. Blow Flies 9 and 10 contained no parasites.

No control blow fly was found to harbour microsporidia.

From the above experiments it can be inferred that a certain number of Calliphora are attacked by *Nosema apis*, and the latter can prove fatal to them if they are ingested by the insect with its food.

(ii) Crane Flies (*Tipula oleracea*). Two crane flies were noticed sucking the viscera of bees prepared for microscopical examination. The crane flies were captured, but refused to feed in captivity and died two days after capture. When dissected, a few young stages of *Nosema apis* were found. Crane flies caught in the open and used as controls showed no trace of microsporidian infection.

(iii) Sheep Keds (*Melophagus ovinus*). All the insects previously mentioned were able to acquire spores of *Nosema apis* naturally by means of their food, though the number of cases occurring in the open may not be very considerable. During the course of this investigation, a number of healthy sheep keds, *Melophagus ovinus*, from a prize Southdown flock, were sent to us. Though it was not very probable that these insects could obtain Nosema spores in nature, it was considered that it might be of

interest to see what effect, if any, was produced on them by *Nosema apis*. In order to ensure the ingestion of Nosema spores by the keds, spores from the guts of bees were smeared on to a limited area of the forearm of one of the writers and six keds were fed on this small area. When wounds were made by the bites, additional small drops of infected excrement were placed on them, and through the layer of spore-containing material the keds had to force their proboscides to get blood. Some of the keds after sampling the sweet excrement slowly sucked it up before taking much blood. All six fed well, the process taking nearly an hour in every case. Six control keds were fed on a carefully disinfected area of the other arm of the experimenter. The next day the treated keds were alive but not active, as it was a very cold day. They were fed again in the same way, all feeding well, but three being particularly greedy. A third feed was given on the next day, when the three weaker feeders of the previous day also fed slightly. Examination of the excrement of these three showed that they contained a fungus, as reported by one of us in 1910.* The other three showed no fungus in their faeces. At noon on the fourth day after the first feed, the three keds containing fungus were dead. Microscopical investigation showed that they contained large quantities of fungus in their Malpighian tubules and young stages of *Nosema apis* in their mid guts. Soon after mid-day, the remaining keds were noticed to be much more feeble, and between 3 p.m. and 9 p.m. that day all three died. Examination of fresh preparations of them showed the presence of meronts and empty sporocysts of *Nosema apis* in the gut contents. Stained preparations were also made and were confirmatory of the fresh preparations; they also contained young spores. When the control keds were dissected, no form of *Nosema apis* was observed in any of them. From this experiment, it is shown that Hippoboscid flies, *Melophagus ovinus*, became infected when supplied with spores of *Nosema apis*, which underwent developmental changes in their bodies.

The Glossinae or tsetse flies resemble the Hippoboscidae in their

* Porter, A. (1910). The Structure and Life-history of *Crithidia melophagia* (Flu), an Endoparasite of the Sheep Ked, *Melophagus ovinus*. Quart. Journ. Microsc. Sci., LV, pp. 189-224. Two plates.

mode of reproduction and blood-sucking habit. As is well known, the Glossinae are the flies that transmit sleeping sickness or trypanosomiasis of men and animals. We would suggest that a search should be made in the various Glossinae by competent observers, who are well versed by practical experience in the structure and life history of Microsporidia, for parasites allied to the Nosema so destructive to bees, and pathogenic also to mason bees, wasps, and the various Lepidoptera and Diptera cited above. Should such a pathogenic Microsporidian be found as a hyper-parasite in Glossinae, it would be a forward step in the solving of the problem of sleeping sickness.

III. SUMMARY AND CONCLUSIONS

1. *Nosema apis* has been proved pathogenic to Hymenoptera other than bees. It can multiply in the food canals of humble bees, mason bees and wasps, and can bring about the deaths of the hosts.

2. Contamination of plants with infected excrement occurs in the neighbourhood of badly infected hives. Such contaminated food is pathogenic to the larvae of cabbage white butterflies, cinnabar moths and gooseberry moths, in which *Nosema apis* produces destruction of the tissue of the food canal in the same way as in bees. Both imagines and larvae of these insects became infected with microsporidiosis when supplied with food contaminated with Nosema spores.

3. *Calliphora erythrocephala*. The blow fly becomes infected naturally by ingesting Nosema spores contained in the sweet excrement of bees. This infection has been repeated experimentally. Crane flies may also become infected.

4. A member of the Hippoboscidae, *Melophagus ovinus*, has been infected successfully with *Nosema apis*, which is pathogenic to the sheep ked. It is suggested that research be made by competent observers among the Glossinae for Microsporidian parasites allied to the Nosema of bees, and, possibly, equally pathogenic to the tse-tse flies that may harbour them.

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ON CERTAIN MOSQUITOS OF THE GENERA *BANKSINELLA*, THEOBALD, AND *TAENIORHYNCHUS*, ARRIBALZAGA

BY

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(*Received for publication* 28 *November*, 1913)

During the past few weeks I have made a critical examination of the male genital armatures of numerous species of African mosquitos and, in certain instances, somewhat interesting results have been obtained from their subsequent study. These studies are more especially connected with the affinities of certain obscure species and in reference also to the synonymy adopted by other students of this group of blood-sucking insects.

Before dealing with the specific characters, it would perhaps not be out of place in this paper, if I draw attention to the fact that the structure of the male genitalia does not support the separation of the pale coloured African species of *Chrysoconops*, so defined by Theobald, from the genus *Taeniorhynchus*. The armatures of typical examples of the latter genus compared with those of the African *Chrysoconops* are so essentially similar that I consider them to be congeneric.

BANKSINELLA PALPALE (Newstead).

Neomelanoconion palpale Newstead. *Ann. Trop. Med. and Parasit.*, I, p. 31 (1907):

Banksinella luteolateralis, Edwards (*nec* Theob.). *Bull. Ent Res.*, III, p. 6 (1912).

This species was originally described by Newstead (*l.c.*) from a single male collected by Drs. Dutton and Todd at Boma, Congo Free State. Unfortunately, however, the specimen was somewhat rubbed and therefore certain of the more important characters rendered obscure. Last year the type was submitted to

Mr. Edwards, of the British Museum, for examination, who subsequently placed the species as a synonym of *B. luteolateralis*, Theob. Some time ago a perfect male example* of an apparently new species was received from Broomassie, Ashanti. The armature of this specimen was prepared for microscopical examination, and, on comparison with a similar preparation of the type of *N. palpale*, was at once seen to be the same. Newstead's species is therefore a valid one, and must be raised to specific rank.

Male genitalia (fig. 1).†

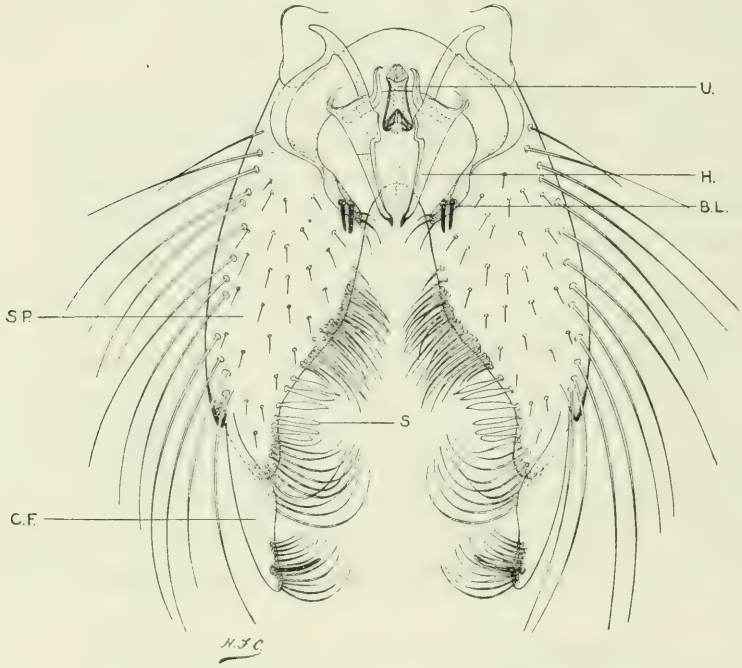

Fig. 1A. Male genitalia of *Banksinella palpale*, Newst. × 160; s = lanceolate spines

Fig. 1B. Inner lateral view of right clasp filament (c.f.) of *B. palpale*, Newst. × 240

Side pieces relatively large, broad posteriorly, narrow and rod-like distally, the internal edges being somewhat strongly curved, as shown in the figure. The latter bear numerous fine hairs in the central region, extending from each side towards the middle line,

* Mr. Edwards has kindly examined this specimen for me.

† In the designation of the parts I have followed Messrs. Howard, Dyar and Knab and the main lettering in all the figures is as follows :—
s.p. = side piece; c.f. = clasp filament; u = unci; h = harpe; b.l. = basal lobe of side piece.

and several long, markedly curved ones on the apical lobe-like projection; between these groups of hairs are situated six or seven lanceolate spines (fig. 1 *s*), but the number of these appears to vary, since in the specimen from Ashanti only three are visible. Clasp filament comparatively short and stout, expanding slightly on the inner side near the middle, tapering off towards the apex, and bearing numerous curved hairs and a large conspicuous, slightly curved tooth-like projection in this region. The inner lateral surface of this organ (fig. 1 *b*) is considerably broader, with a blunt and rounded apex; the hairs and tooth previously mentioned, arise from the apical portion of this surface and extend towards the middle line. Basal lobes small, each bearing two distinct spines or teeth, and a few

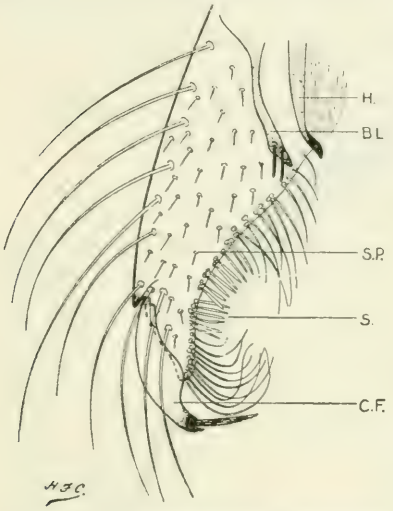

FIG. 2A. Left side-piece and appendages of *Banksinella luteolateralis*, Theob. × 160; s = lanceolate spines.

FIG. 2B. Left clasp filament enlarged (× 240)

delicate hairs. Harpes well developed, the apical extremity pointed, forming a single tooth. Harpagones absent. Unci of complex structure bearing three pairs of comparatively large, inwardly and ventrally directed, teeth on the apical ventral margin; these teeth increase proportionately in size, the lowermost pair being the smallest.

The genital armature of *B. luteolateralis* (fig. 2) is very similar to that of the previous species as regards the structure of the basal parts, and general form of the side pieces. The clasp filaments

(fig. 2 *b*) provide, perhaps, the most distinctive character. These are of a somewhat different shape, and are completely devoid of hairs at the apical extremity; the tooth, also, is distinctly larger. The internal edges of the side pieces bear similar arrangements of hairs and spines as in *B. palpale*, but the hairs of the central region are less numerous, and the lanceolate spines are smaller and about fifteen in number.

As previously mentioned, the type of *Banksinella palpale* was in a somewhat damaged condition when described, and I therefore thought it desirable to add a few notes on the specific characters of the male.

Head: The narrow, curved scales occupy a narrower median area than in *B. luteolateralis*, Theob., and the pale coloured region is formed to a considerable extent by cream coloured flat scales.

FIG. 3. (*a*) Male palpus of *Banksinella palpale*, Newst.; (*b*) Male palpus of *Banksinella luteolateralis*, Theob.; × 36.

Palpi with the second or terminal segment relatively much shorter than in *B. luteolateralis*; the basal segment is nearly three and one-third times the length of the apical segment, whereas in *B. luteolateralis* it is approximately two and one-third times as long. *Proboscis* sometimes showing an ill-defined yellowish band.

Thorax: Clothed with golden, narrow-curved scales laterally, dark curved scales and a few scattered golden ones in the central region—similar to the ornamentation usually met with in this genus.

Abdomen: First two segments dark, unbanded; third and fourth with basal lateral cream coloured spots, especially noticeable on the latter; fifth, sixth and seventh segments* with well-marked yellowish-white basal bands. In the male of *B. luteolateralis* the first and second segments usually possess a few pale scales on the median area, the others being adorned with basal pale bands.

Wings: Very similar to those of *B. luteolateralis*.

Legs: Brown, the femora and tibiae being pale on the ventral surface; tibiae with apical knee-spots, well marked in the hind pair of legs.

TAENIORHYNCHUS MACULIPENNIS (Theobald).

Chrysoconops maculipennis, Theobald. *Novae Culicidae*, Part I, p. 27, April, 1911.

This species, described by Theobald from Uganda, has recently been placed as a synonym of *Taeniorhynchus* (*Chrysoconops*) *annettii*, Theob., by Mr. Edwards. Through the kindness of Mr. H. H. King, of the Wellcome Laboratories, a male example has been received by the School, and an examination of the genitalia at once proves the species to be a valid one. Mr. Edwards writes that he has now examined preparations of the armature and is in a position to confirm the above statement.

Male genitalia (fig. 4.)

Side pieces large, gradually tapering to a broadly rounded apex. Clasp filaments long and relatively narrow, each bearing two short closely appressed teeth at the tip. The apical third bears four delicate hairs, and is deeply constricted at its base, on the lower side. Basal lobes large, each with a pair of dark rod-like appendages; one of the latter, in each case, is a very conspicuous, stout, elongated structure, the other much narrower and slightly shorter. The larger rod-like process is evidently composed of three or four fused spines or modified hairs, the smaller rod of one only.

* The last few segments are necessarily wanting, as they were removed for microscopical examination.

Harpes well marked, with five comparatively large teeth at the extremities. Harpagones absent. Unci as depicted (fig. 4 *u*), bearing numerous minute teeth on the apical, dorsal and ventral edges.

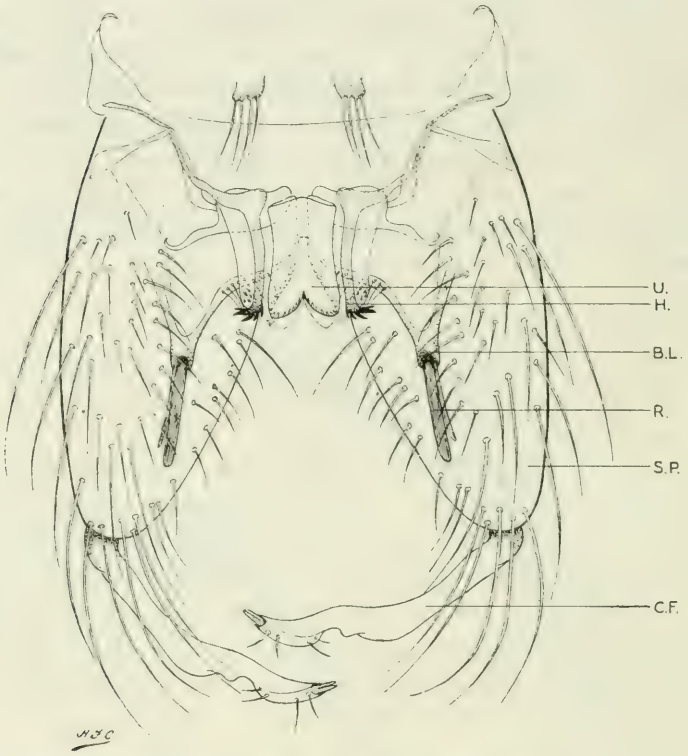

FIG. 4 Male genitalia of *Taeniorhynchus maculipennis*, Theob.; × 160.

Genital armature of T. annettii (fig. 5).

The main point of difference between this and the previous species is in the structure of the clasp filaments. These are of very peculiar form, and an idea of their shape can best be gained from the figures. The apical portion is broad and very deeply cleft on the ventral surface; it bears a few delicate hairs and three teeth, closely pressed together, at the tip.

The basal parts closely resemble those of *T. maculipennis*, although the harpes appear to be more elongated (*vide* fig.) This, however, may be due to displacement of the parts in mounting,

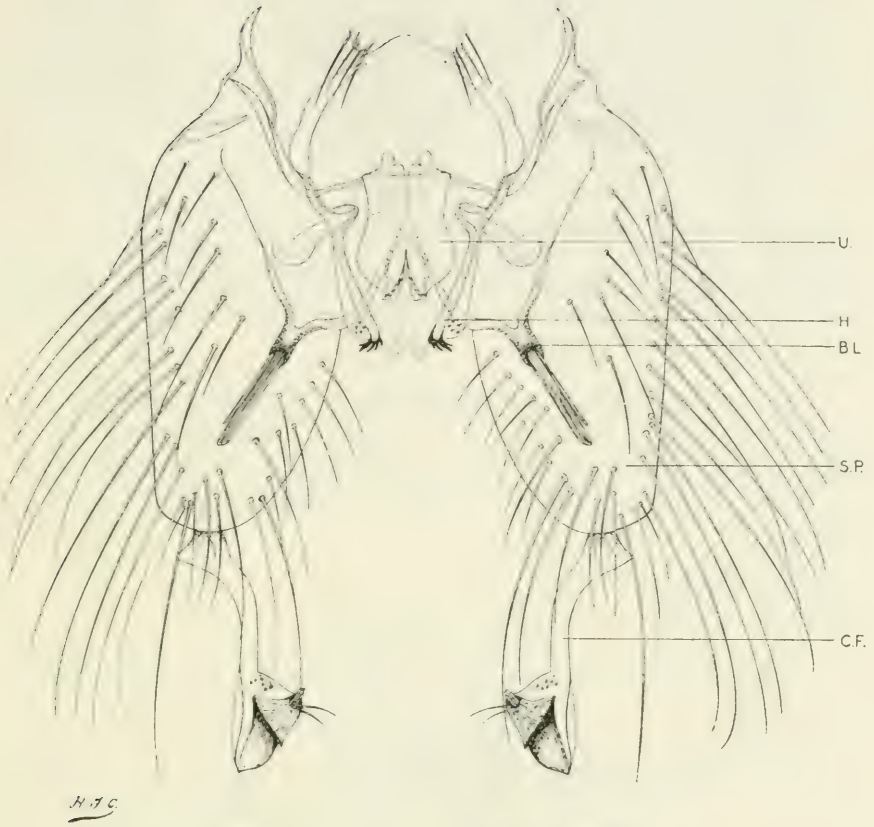

FIG. 5A. Male genitalia of *Taeniorhynchus annettii*, Theob.; × 160.

FIG. 5B. Right clasp filament of *T. annettii*, mounted under pressure; × 240.

but, owing to lack of material, no definite statement can be made. The identification of these two species by other means is a matter of no little difficulty, and, unfortunately, the material at my disposal is insufficient to enable me to give any constant and well-defined characters for distinguishing purposes.

TAENIORHYNCHUS METALLICUS (Theobald).

Culex metallicus, Theobald. *Mon. Cul.*, II, p. 63 (1901).

Banksinella metallicus (Theobald). *Mon. Cul.*, V, p. 408 (1910).
Taeniorhynchus violaceus, Theobald. *Third Report Wellcome Labs.*, p. 262 (1908).

The examination of preparations of the male armature of authenticated specimens of *T. metallicus* from various parts of Africa, and of *T. violaceus* from the Sudan (Mr. H. H. King) show that the above synonymy, previously proposed by Mr. Edwards, is correct.

Male genitalia (fig. 6).

FIG. 6. Male genitalia of *Taeniorhynchus metallicus*, Theob.; × 160.

Side pieces sub-cylindrical, adorned with the usual stout and slender hairs, and bearing basal lobes with their appendages, which greatly resemble those of the two above-mentioned species. Clasp filament very characteristic, the apical two-thirds being expanded for part of its length, then tapering gradually to the tip, on which is situated a very short, stout tooth; the lower side of the apical half is sharply re-curved, forming a distinct ridge. Harpes apparently provided with three teeth. Harpagones absent. Unci with a series of eight or nine small teeth on the ventral basal edges, and four larger teeth at the apex.

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NEW CULICIDAE FROM THE SUDAN

BY

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(*Received for publication* 24 *November*, 1913)

Amongst a large collection of Culicidae sent me by Mr. Harold King, from the Sudan, the following undescribed species have so far been found.

A considerable number of *Uranotaenias* and others have as yet only been partially examined, and will be reported upon later. The types of these new species are in the collection of the Liverpool School of Tropical Medicine.

MUCIDUS NIGERRIMUS, nov. sp.

♂. *Head*: Brown, covered with mealy grey scales, a narrow white border around the large dark eyes, a tuft of long grey curled scales and long, thin, straight ones projecting forwards between them. *Antennae* brown, with deep brown plume hairs; basal segment bright brown, with small flat white scales. *Palpi* deep brown, with black scales irregularly disposed and scattered white scales, especially at the junctions of the last two segments, forming distinct bands; plume hairs deep brown, apical segment slightly longer than the penultimate; longer than the proboscis by about two-thirds of the last segment. *Proboscis* with scattered dark and light scales, as in the palpi, a distinct joint about the middle, the apical half being thinner than the basal.

Thorax: Brown, clothed with irregular scattered grey scales, most dense in the median area and around the space before the scutellum and across the thorax between the wings. *Scutellum* very densely clothed with loose grey scales; the scantier scaled areas have long, thin, narrow-curved, pale scales, not typical *Mucidus* ones, which also form a dense scaled median area in front. *Pleurae* very deep brown, with patches of flat white scales. *Metanotum* rich brown.

Abdomen: Deep brown, clothed with alternate patches of black and white scales, forming marked black and white outstanding lateral patches; hairs dense, pale golden; the segments dorsally, with flat, loose white scales at the base of the segments, in the middle, with mixed dark and creamy ones apically and at the sides more evenly disposed; the apical segments more white scaled, the last but one with two black spots. Basal lobes of genitalia, with black scales; claspers long, curved, dark.

Legs: Fore femora thin, with dark and pale scales, the former predominate, apex white; fore tibiae with a dull white basal band, followed by a black area with outstanding scales and then a snow-white area with outstanding scales; tarsi thin, yellow, slightly darkened apically; mid legs with the femora with dense outstanding scales, black predominating, with three narrow white bands and white apex; tibiae with white basal and apical bands, black in middle, with a narrow median white band, very shaggy; tarsi thin and yellow; hind legs with femora and tibiae as in the mid, but not quite so shaggy or dark; first tarsal black scaled, with narrow basal white band, yellowish at apex, very shaggy; remaining tarsals white basally, yellow apically, scales more or less outstanding; the white predominate on the last three segments; hind ungues equal and uniserrate, fore and mid with the larger claw bidentate, the smaller unidentate (viewed in one direction the second or basal tooth of the larger claw seems to stand out laterally, looking like three claws).

FIG. 1. *Mucidus nigerrimus*. n. sp. ♂
Fore ungues

Wings: Mostly dark scales, with a few scattered white ones; first fork-cell considerably longer and narrower that the second, its base nearer the base of the wing, its stem as long as the cell, stem

of the second longer than the cell; third long vein very close to the second; cross-veins thick, clouded, all three close together; fringe with eight white spots; the scanty dark scales give the wings a spotted appearance. *Halteres* yellow, knob black at the apex.

Length. 7 mm.

Habitat. Wadelai, Lado District, Sudan. (H. King, 8/6/11.)

Observations. Described from a perfect male taken from undergrowth around trees on a grassy slope near the Nile. It was pointed out as being a distinct species by Mr. King. It is a very dark species, easily distinguished by the leg ornamentation.

Chrysoconops nocturnus, nov. sp.

Head golden-yellow; proboscis and palpi golden-yellow, black at their apices. Thorax shiny black, with scattered golden scales; pleurae mostly brown, a long yellowish area before wing roots. Abdomen entirely golden-yellow. Legs golden-yellow, femora with some scattered dark scales and narrow dark apical bands; fore and mid tibiae with scattered dark scales and a small dark apical band; hind tibiae with narrow basal and broad median and apical dark bands; fore and mid tarsi golden-yellow, unbanded, last two tarsals dark; hind legs with metatarsi, first, second and third tarsals with broad apical black bands, last all dark. Wings yellowish, all the veins with dusky brown and dull yellowish scales. Male palpi with three dark rings, legs darker than female and traces of dark apical abdominal bands.

♀. *Head*: Integument greyish, clothed with golden-yellowish narrow-curved scales and dark, thin, upright, forked scales; eyes silvery. *Antennae* brown, basal segment pale. *Palpi* and *proboscis* golden, black scales at their apices, with black chaetae and some scattered black scales on the apical half of the proboscis, also some black scales below, at the base (one specimen shows some scattered dusky scales over the palpi).

Thorax: Shiny black, with scattered golden-yellow, thin, narrow-curved scales, and black chaetae; prothoracic lobes projecting, golden-yellow, roundish and mammillate. *Scutellum* black, shiny, with golden narrow-curved scales and dark border bristles. *Metanotum* black. *Pleurae* deep brown with some

patches of flat white scales and a yellow, longish area at the base of the wings; a short blunt process appears on each side of the front region of the mesonotum. (Fig. 2. B.)

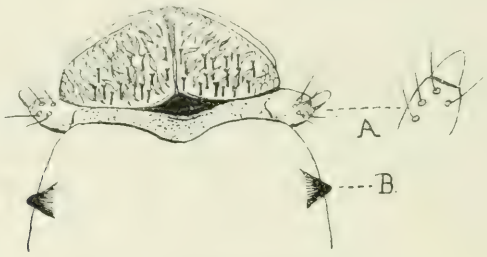

Fig. 2. *Chrysoconops nocturnus*. n. sp.
A. = Prothoracic lobe. B. = Mesonotal process.

Abdomen: Steely, entirely clothed with bright golden-yellow scales and yellow hairs; venter golden yellow.

Legs: Golden-yellow, femora with some scattered black scales and black apices, fore and mid tibiae with scattered black scales and black apices, hind with narrow dark basal rings, a broad black median and apical rings; fore and mid tarsals unbanded, the last two dusky; hind tarsals with broad apical black bands; chaetae dark; ungues dark, equal and simple.

Wings: Yellowish with scattered dusky and yellow scales, appearing mainly dusky in certain lights; first fork-cell longer and narrower than the second, its base nearer the base of the wing, its stem about one-third the length of the cell; stem of the second fork-cell not quite so long as the cell; posterior cross-vein about twice its own length distant from the mid.

Halteres pale yellow.

Length. 5 mm.

♂. *Palpi* with the last two segments nearly equal, the apical one mostly black, apex of the next two dark; scanty hair tufts, brown. *Antennae* banded yellow and brown with rich brown plume hairs.

Legs: As in ♀, but more dark scales above on the femora and tibiae.

Abdomen: With some dark metallic scales on the apices of the segments, almost forming bands, apical segment dark, with dark

chaetae and dark claspers. Fore and mid ungues very unequal, the larger uniserrate; hind ungues rather long, curved and simple, all black.

Length. 5 mm.

Habitat. Bier Terab (Shambe to Wau road), Bahr-el-Ghazal Province; Lau (Shambe to Wau road); Lau to Ateiba (Shambe to Wau road) and Mayo to Melangot (Naam River), Bahr-el-Ghazal Province. (H. King, 30/12/10, 3/1/11, 10/1/11.)

Observations. Described from three ♀'s and one ♂. The specimens were taken by Mr. King in a rest house after dark and out of doors after dark. It is a vicious blood-sucker. This species comes near *C. nigrithorax*, Theob., which it superficially resembles, but it is distinct, as pointed out by Mr. King. The tibiae are not all black, as in *C. nigrithorax*; the proboscis has a few black scales at the base, but is not deep black on the apical half, as in *C. nigrithorax*. The marked prothoracic lobes also differ, and the blunt lateral processes on the mesonotum are also characteristic.

REEDOMYIA SUDANENSIS, nov. sp.

Head dark, a black patch and then a pale patch on each side, middle with golden scales and black upright scales; eyes silvery and black; palpi, proboscis and antennae deep brown. Thorax deep rich brown with scattered small golden scales; scutellum silvery white; pleurae blackish-brown to brown with white puncta. Abdomen deep blackish brown, unbanded, with small basal lateral white spots; venter with basal yellow scales, the segments with blackish apical borders. Legs deep brown, unbanded, hind femora and tibiae with apical white spots, seen also in fore and mid legs to some extent; chaetae golden.

♀. *Head*: Black and shiny with scattered narrow-curved pale golden scales in the middle, with black upright forked scales, a patch of flat black scales at the sides and then flat creamy white ones. *Eyes* silvery around the upper border and down to the antennae. *Palpi* black. *Proboscis* deep brown. *Antennae* deep brown with pale pubescence and dark hairs, basal segment and base of second paler.

Thorax: Deep rich brown, with small narrow-curved scattered

golden scales, a small patch of broader creamy ones in front of the root of each wing; chaetae dark. *Scutellum* with flat silvery white scales and numerous rather irregular dark border bristles. *Metanotum* dark with a greyish sheen. *Pleurae* black, with patches of white scales.

Abdomen: Black, unbanded, with small basal white lateral spots, dark border bristles with dull golden apical reflections; venter with basal creamy yellow areas, dark apical ones.

Legs: Deep brownish-black, unbanded, apices of femora and tibiae white, especially in the hind legs, chaetae golden, showing markedly against the dark legs; ungues of fore and mid legs equal and uniserrate, of hind equal and simple.

Wings: First fork-cell longer and narrower than the second, its base nearer the base of the wing than that of the second, its stem about half the length of the cell; stem of the second fork-cell not quite as long as the cell; posterior cross-vein about its own length distant from the mid.

Halteres with pale stems and fuscous knobs, with some pale scales on the latter.

Length. 4 to 4·5 mm.

Habitat. Yidu, Lado District, two ♀'s; Khor Nambiri, Lado District, one ♀; Nyumbe, Lado District, one ♀; Such River, one ♀; 12 to 14 miles from Hierallah on road to Bundle, Lado District. (H. King, 22/3/11, 2/4/11, 27 and 31/5/11.)

Observations. Described from six ♀'s. The marked head adornment is very noticeable. In one ♀ there are traces of the thoracic scales forming two dorsal spots, but the deep rich brown with fine golden scale dusting is most noticeable in all six specimens. It comes near *R. bipunctata*, Theob., but the scutellum is snowy white, not pale yellowish, and the thorax has golden, not bronzy scales; from *R. biannulata*, Theob., it can be told by the apex of the abodmen not being white and from *R. neobiannulata*, Theob., by having no basal white abdominal bands.

Mr. King's notes are as follows:—

'Khor Nambiri (Kapei to Yei), Lado District, 2/4/11. A small khor containing pools, and shaded by trees and bushes.'

'Nyumbe, Lado District, 27/5/11. Amongst undergrowth in a grove by a khor.'

'Yidu, Lado District, 31/5/11. From undergrowth on the bank of a typical *Glossina palpalis* khor.'

'12-14 miles from Hierallah on the road to Bundle, Lado District, 22/3/11. In a deep, heavy timbered ravine.'

KINGIA MACULOABDOMINALIS, nov. sp.

Head black, silvery white on middle and sides; proboscis black; palpi black, snow white tips; antennae deep brown, black at base with white scales; eyes silvery. Thorax rich deep brown, a large patch of snowy white on each side, a small one over the wings, and lines of yellowish scales posteriorly; scutellum snowy white in some lights, grey in others; pleurae deep brown with white puncta. Abdomen deep black, unbanded, 5th to 7th segments with silvery white median spots, and with basal lateral white spots. Legs dark brown, femora with traces of two white spots; first and second tarsals only with narrow basal pale bands (fore and mid legs).

♀. *Head*: Black, clothed with flat black scales, flat white ones in the middle and a patch of white ones on each side; chaetae black. *Palpi* black scaled with snow white scales apically. *Proboscis* black. *Antennae* deep brown, the basal segment with flat white scales, the second segment with outstanding dark scales, hairs deep brown.

Thorax: Shiny black with deep bronzy black, narrow-curved scales, two large patches of snow white flat scales about the middle of the mesonotum, a small patch of narrow-curved white ones just in front of the roots of the wings, a short line of pale creamy scales running up to the bare space before the scutellum, and a line of yellowish scales on each side parallel to it and reaching the scutellum; chaetae black. *Scutellum* black, the flat scales are glassy and snow white in some lights, grey to almost black in others; border bristles blackish brown, four to the mid lobe. *Metanotum* deep chestnut-brown. *Pleurae* deep brown with silvery white puncta.

Abdomen: Black with basal snow white spots, the fifth with two median silvery white spots, the sixth with one median snow white spot, the seventh with the spot almost basal, a small one also on the apex; border bristles deep brown; venter dark brown.

Legs: Blackish-brown, femora with two white spots; first and second tarsals of fore and mid legs with basal creamy white bands, others dark in fore and mid legs (hind legs missing); ungues equal and uniserrate.

Wings: First fork-cell longer and narrower than the second, its stem more than half the length of the cell, stem of the second fork-cell nearly as long as the cell; posterior cross-vein rather more than its own length distant from the mid; scales brown, rather large.

Length. 4 mm.

Habitat. Khor Kokbwa (Yei to Abba), Lado district. (H. King, 13/4/11.)

Observations. Described from a single ♀ taken by a rocky, shady stream; the hind legs missing. A very marked species readily distinguished by the thoracic and abdominal adornment.

AEDIMORPHUS QUINQUEPUNCTATA, nov. sp.

Head black and silvery white; palpi, proboscis and antennae deep brown. Thorax rich brown with four silvery white spots and a small fifth white patch before the bare space in front of the scutellum, which is also silvery white scaled; pleurae deep brown with silvery white puncta. Abdomen dusky black, unbanded, with basal lateral silvery white spots; venter with basal silvery white lateral spots also. Legs dark, unbanded, with venter of femora pale at the base, especially on the hind legs where most of the venter is pale, femora of hind legs, each with a snow white apex and a spot before the apex.

♀. *Head*: Black with a small median area of dull creamy narrow-curved scales, with black upright forked scales, a patch of flat snow white scales on each side in front, then flat black scales, then white and then black ones again. *Clypeus*, *proboscis*, *palpi* and *antennae* deep brown to almost black.

Thorax: Dull black with small, narrow-curved, dull brown scales and four patches of small flat snow white scales, two in front near the head, two in the middle of the mesonotum, also a fifth patch before the bare space in front of the scutellum, and a small spot below in front of each wing; chaetae black. *Scutellum*

black with snow white flat scales forming three patches, border-bristles black, four large ones to the mid lobe. *Metanotum* deep black; pleurae rich brown with six snow white puncta.

Abdomen: Black with dull golden border bristles and basal snow white lateral patches, the scales somewhat outstanding; venter black with basal snow white lateral spots.

Legs: Almost black, femora and tibiae with apical white spots most prominent on the hind legs, each of the hind femora with a round snow white spot near the apex and whitish on most of the venter; ungues of fore and mid legs equal and uniserrate, hind equal and simple.

Wings: With a white scaled spot at the base and with the first fork-cell slightly longer, but no narrower than the second fork-cell, its stem more than half the length of the cell; stem of the second fork-cell about two-thirds the length of the cell; posterior cross-vein less than its own length distant from the mid; sixth long vein markedly curved. *Halteres* with ochreous stem and ochreous and dusky knobs.

Length. 3·5 mm.

Habitat. Alenga and Matalee, Lado District. (11/5/11 and 28/5/11, H. King.)

Observations. Described from two ♀'s. It comes near *Aedimorphus punctithorax*, Theob., but differs in having five, not six, thoracic white spots.

CULICELSA CENTROPUNCTATA, nov. sp.

Head brown with pale scales and two dark areas on each side separated by a line of white; palpi, proboscis and antennae deep brown. Thorax with brown and silvery white scales, the latter forming two spots in front, a median and more or less pronounced posterior area; scutellum entirely white scaled. Abdomen black with median white basal patches and basal white lateral spots; venter mostly creamy scaled. Legs black, with pale chaetae, especially marked on the tibiae, femora and tibiae with apical white to yellow bands, tarsi with narrow basal white bands to all the hind segments, and all but the last two in the fore and mid.

♀. *Head*: Clothed with almost white narrow-curved scales in the middle and dark, thick, upright forked scales, then a few flat

white scales, then a black patch, then white, then black again; chaetae long and deep brown, except for some golden ones between the eyes. *Palpi*, *proboscis* and *clypeus* almost black. *Antennae* black with pale internodes, basal segment dark and testaceous, the second with flat black scales; verticillate hairs black.

Thorax: Deep brown with mixed brown and silvery white narrow-curved scales, the latter forming two spots in front, a border around the mesothorax near the head, denser behind, and as a line above each wing; chaetae long and black. *Scutellum* with narrow white curved scales. *Metathorax* black. *Pleurae* deep brown with patches of flat white scales.

Abdomen: Black, the segments with median basal patches of white scales, and patches of basal lateral white scales which become median apically, posterior border bristles golden; venter mainly creamy scaled.

Legs: Almost black, femora and tibiae with apical pale spots, base of first and second fore and mid tarsals with white bands; in the hind legs all the tarsals have basal white bands, bristles dark and golden; fore and mid ungues equal and uniserrate, hind equal and simple.

Wings: With short fork-cells, the first longer and narrower than the second, its base a little nearer the base of the wing, its stem nearly as long as the cell; stem of the second longer than the cell; posterior cross-vein twice its own length distant from the mid. *Halteres* all pale ochreous.

Length. 4 mm.

♂. *Head*: Pale scaled with two dark patches of flat scales surrounded by white flat scales. *Palpi* a little longer than the proboscis, black, with the last two segments nearly equal and with small basal white bands, a creamy band at the base of the third segment and a pale area at their base, on the last two segments are brown hair-tufts on one side, and on the apex of the penultimate is a long golden chaeta. *Antennae* banded brown and grey, plume hairs flaxen brown.

Thorax, *abdomen and legs* as in the ♀; fore and mid ungues, unequal, uniserrate, hind equal and simple.

Length. 4·5 mm.

Habitat. Alcholi, Lado District and River Mewri, Mongolla Province. (25/5/11, ♀; 21/3/11, ♂, H. King.)

Observations. Described from a perfect ♂ and ♀, although taken in different places undoubtedly the same species. It is a most marked species and is related to those so far placed in the somewhat obscure genus *Culicelsa*, Felt, obscure only for the fact that we cannot fix any definite characters, yet all the species included have a very marked similar appearance.

Heptaphlebomyia kingii, nov. sp.

Head black with pallid scanty scales, proboscis ochreous black at the base and apex; antennae and palpi dark brown. Thorax rich brown, somewhat paler in the middle, two snow white spots in front near the head and two on the middle of the mesothorax; scutellum snow white scaled; pleurae deep brown with snow white puncta.

Abdomen deep brown, unbanded, with basal lateral white spots; venter with pale basal bands. Legs unbanded, deep brown, femora and tibiae with apical white spots. Wings with seventh vein very close to the wing border.

♀. *Head*: Black with pale creamy, narrow-curved scales in the middle, becoming white in front, creamy flat lateral scales, white at eye border near the white narrow ones; upright forked scales black. *Antennae*, *palpi* and *clypeus* almost black. *Proboscis* ochreous brown, black at the base and apex.

Thorax: Black with narrow-curved bronzy brown scales, two median bare parallel lines, four round spots of snow white narrow-curved scales, two in front near the head, two somewhat larger and wider apart about the middle of the thorax; the scales become paler towards the scutellum, being pale golden in some lights; chaetae brown. *Scutellum* black with dense snowy white curved scales, deep brown border bristles, six to the mid lobe; metanotum black with grey reflections. *Pleurae* deep brown with patches of rather long, flat, white scales.

Abdomen: Steely, clothed with black scales, with small snow white basal lateral spots; border bristles brown with golden reflections apically; venter black with basal white bands.

Legs: Dark brown, unbanded, femora pale at the base, apices of femora and tibiae white on the hind legs, traces in the mid, scarcely perceptible in the front pair; ungues equal and simple.

Wings: With brown scales; first fork-cell considerably longer and a little narrower than the second fork-cell, its base nearer the base of the wing, its stem less than one-fourth the length of the cell, stem of the second posterior not quite as long as the cell; posterior cross-vein not quite twice its own length distant from the mid; the seventh scaled vein close to the inner border of the wing. *Halteres* pale with fuscous knobs.

Length. 4 mm.

Habitat. Nyumbe, Lado District; Alenga, Lado District. (27/5/11, Nyumbe; 28/5/11, Alenga.)

Observations. Described from two perfect ♀'s. It can be distinguished from the allied *H. argenteopunctata*, Ventrillon, by the abdomen having only small basal lateral spots and not ornamented as in Ventrillon's species from Madagascar; the antennae are also black in the ♀, not yellowish, and the thoracic adornment differs. The scaled seventh vein is well marked in both ♀'s. One was taken from a small hole, containing water, in a tree, the other from tall grass near a khor.

THE PROBABLE IDENTITY OF *TRYPANOSOMA CONGOLENSE* (BRODEN) AND *T. NANUM* (LAVERAN)

BY

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(*Received for publication* 28 *November*, 1913)

In our paper on the identification of the more important mammalian trypanosomes, we have regarded *T. dimorphon* (sensu Laveran and Mesnil), *T. confusum* (Montgomery and Kinghorn) and *T. pecorum* (Bruce) as synonymous with *T. congolense*, which was first described by Broden in 1904. In the same year Laveran described a similar parasite found by Balfour in the Sudan, under the name of *T. nanum*. These parasites are identical morphologically in that they are both short aflagellar trypanosomes measuring 8 to 19μ in length. The sole distinguishing feature is their effect on small laboratory animals, *T. congolense* being described as pathogenic for monkeys, dogs, rabbits, guinea-pigs, rats and mice, while *T. nanum* is considered to be incapable of infecting these animals. The object of this paper is to examine the evidence upon which this distinction is based and to decide whether it is sufficient to warrant such a differentiation.

In previous papers* a description has been given of two trypanosomes which were present in the blood of a naturally infected horse sent over to this country from the Gambia. One of the parasites was unquestionably *T. vivax*: concerning the identity of

* Yorke and Blacklock. The trypanosomes found in two horses naturally infected in the Gambia. Annals of Tropical Medicine & Parasitology, 1911, Vol. V, p. 413.

Blacklock. The trypanosomes found in a horse naturally infected in the Gambia. A double infection. Annals of Tropical Medicine & Parasitology, 1912, Vol. VI, p. 107.

the other there was, however, considerable doubt. Morphologically, this parasite was indistinguishable from *T. dimorphon* (Laveran and Mesnil) and *T. nanum*. As we finally succeeded in infecting small animals with the trypanosome, it was eventually decided that it was *T. dimorphon* (*T. congolense*).

Both the parasites, which were separated fortuitously, one from the other as already described, have been kept in experimental animals for a period of 18 months. The results of artificial passage of the short aflagellar parasite from animal to animal are so interesting and suggestive that we have decided to describe them in some detail.

The sub-inoculations made with this parasite from the time of its isolation until the 51st generation are given in genealogical form in the table. A study of this table reveals two facts, viz.:—

(1) Most of the early inoculations failed to infect, whereas the later were invariably successful.

(2) The course of the infection in the earlier successful cases was chronic, whereas that in the later instances was acute. Thus, if we consider the animals used in the second to the fourth generation, we find that these comprise 8 rats, of which 3 were positive and 5 negative; 4 mice, of which 2 were positive and 2 negative; 3 rabbits, 2 positive and 1 negative; 4 guinea-pigs, 2 positive and 2 negative; and 3 goats, 1 positive and 2 negative.

Again, if the duration of the disease in the earlier rats be compared with that in the later experiments, the contrast is very striking. For example, the average length of life of the first ten rats from the fifth generation to the fourteenth was 88·6 days, whereas that of the last ten rats, comprising the 42nd to the 51st generation, was only 8·6 days.

It is clear, therefore, that by passage of this parasite through laboratory animals the trypanosome has been changed from one of uncertain and chronic pathogenicity to one of great virulence.

This fact seems to us to be one well worthy of remark. That artificial passage of a strain through a series of animals does sometimes alter its virulence for that species is well known. The results*

* Warrington Yorke. On the pathogenicity of a trypanosome from a case of Sleeping Sickness contracted in Rhodesia. Annals of Tropical Medicine & Parasitology, 1910, Vol. IV, p. 351.

obtained by different workers with *T. gambiense* are illustrative of this point.

These observations appear to us to have some significance for the nomenclature of the parasite. *T. congolense* and *T. nanum* are identical morphologically, they are both spread by the same species of tsetse fly and infect the insect in precisely the same manner.

The usual way of deciding with which parasite cattle or antelope known to harbour a short trypanosome in their blood are infected, is by sub-inoculation of rats or some other convenient laboratory animal. As a rule, these animals are not too plentiful in the tropics and one or two must suffice for the diagnosis. That inoculation of one or two small animals may not afford any conclusive evidence as to whether or not the trypanosome is pathogenic, is at once realised from observing the results of the earlier inoculation of the parasite from our horse. Had the number of our experimental animals been limited, we should probably have designated the parasite *T. nanum*. Further experiments, however, showed that the trypanosomes could be made acutely pathogenic to rats.

This opens up the question as to whether there is really any difference between *T. congolense* and *T. nanum*. It is interesting in this connection to refer to the observations of other workers. Writing in 1911 on a short aflagellar trypanosome obtained from ponies naturally infected in Togoland, Weissenborn* states that the parasite was of inconstant virulence. It was most virulent for mice, but slightly so for rabbits, rats and monkeys. Only a small proportion of rats were susceptible, whilst guinea-pigs were absolutely refractory. Morphologically the parasite *T. frobeniusi* closely resembled *T. congolense*.

The Sleeping Sickness Commission of the Royal Society† write: 'If *T. pecorum*, which is usually more or less infective in the monkey, dog and rat, lives for some time in the blood of the goat, it loses its power of infecting other animals. This has given rise to the erroneous idea that a separate species—*T. nanum* exists.'

* Weissenborn, E. Beitrag zur Kenntnis der kurzgeisseligen Trypanosomen. Archiv. f. Schiffs- und Tropen-Hygiene 1911, p. 477.

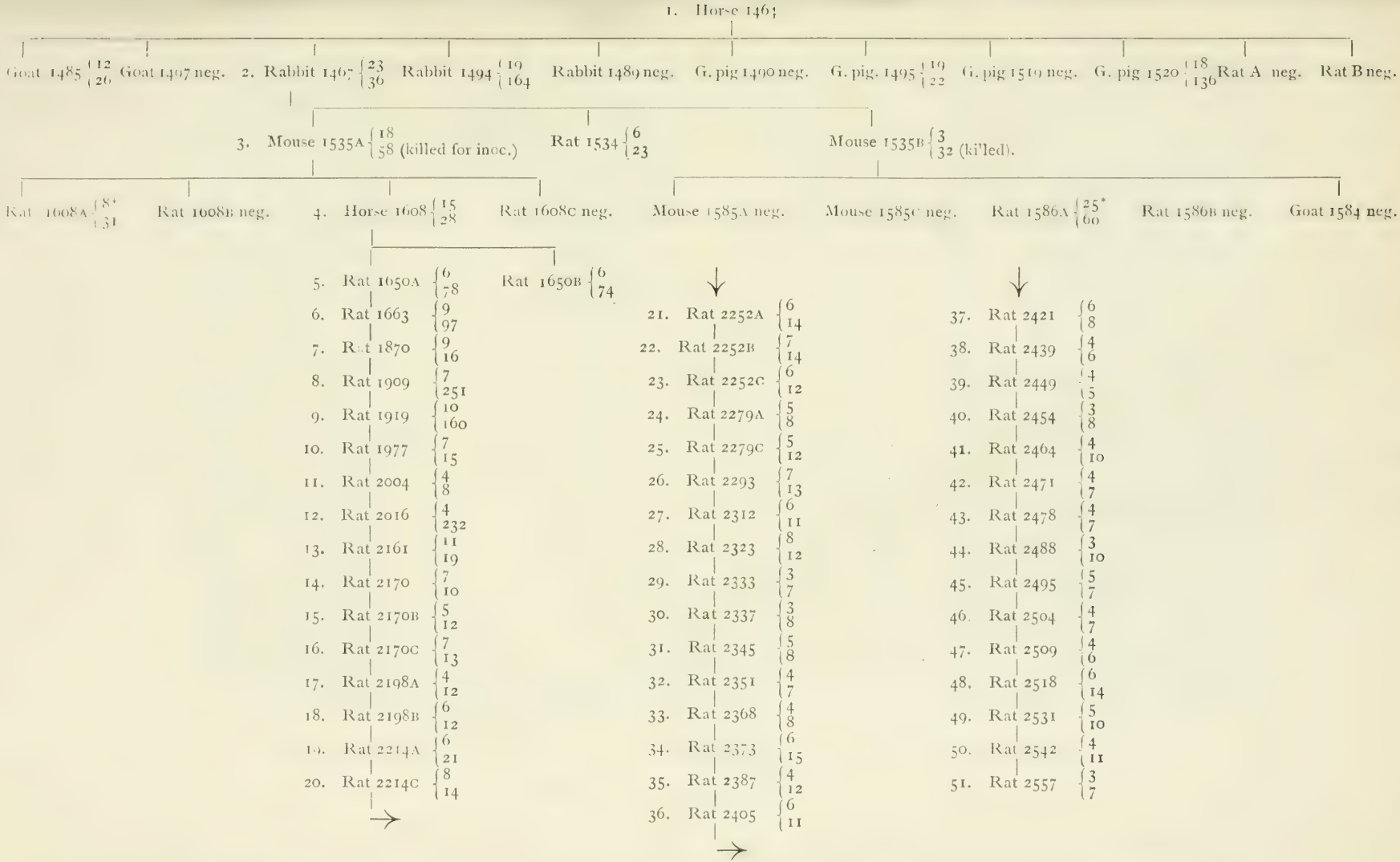
† Bruce, Harvey, Hamerton and Lady Bruce. The susceptibility of various animals to *T. simiae*. Roy. Soc. Proc., 1913, Vol. 87, p. 49.

The Belgian Sleeping Sickness Commission* found that aflagellar trypanosomes from naturally infected dogs will not always infect guinea-pigs and rats.

It is thus evident that workers in the field have found that short aflagellar trypanosomes, morphologically identical with *T. congolense* and *T. nanum*, are of uncertain pathogenicity for the smaller laboratory animals.

As the result of our investigations and of those of the authors mentioned above, we can see no evidence which would justify distinguishing one from the other on the ground of pathogenicity. In the present state of our knowledge we can only conclude that *T. congolense* and *T. nanum* are the same parasite.

* Rodhain, Pons, Vanden Branden, and Bequaert. Rapport sur les Travaux de la Mission Scientifique du Katanga. (Octobre 1910 à Septembre 1912.)



* Parasites appeared on one day only in very small numbers. The upper figures in each bracket indicate incubation, the lower duration.

HERPETOMONAS STRATIOMYIAE, N.SP., A FLAGELLATE PARASITE OF THE FLIES, *STRATIOMYIA CHAMELEON* AND *S. POTAMIDA*, WITH REMARKS ON THE BIOLOGY OF THE HOSTS

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PLATE XLI

CONTENTS

		PAGE
I.	Introduction	609
II.	The Biology of the Host Flies, *Stratiomyia chameleon* and *S. potamida*	610
III.	Material and Methods	612
IV.	The Life-History of *Herpetomonas stratiomyiae*	
	(A) The Herpetomonas in the Larva of the Fly	612
	(B) The Herpetomonad during Pupation	615
	(C) The Herpetomonad in the Imago	616
V.	Mode of Infection	617
VI.	Concluding Remarks	617
VII.	Summary	618
	References	619
	Explanation of Plate	620

I. INTRODUCTION

The interesting flies, *Stratiomyia** *chameleon* and *S. potamida*, are fairly common in certain districts where the drainage of the land is poor and where boggy areas with somewhat rank vegetation occur. Both the larva, pupa and imago of the insects are sometimes parasitised by a small Protozoön, belonging to the genus

* The original generic name of the host flies was *Stratiomys*. Many modern authorities prefer the form *Stratiomyia*, which has been adopted in this paper. The *Stratiomyia* were obtained near Cambridge, and one local name for them is Chameleon flies.

Herpetomonas, and presenting certain differences from other common Herpetomonads that warrant its inclusion as a new species. The chief interest of the parasite lies in its relation to the life-history of the host, and thus a brief note on the biology of the hosts is considered advisable.

We do not propose to enter into great cytological detail regarding the structure of the flagellate, nor to engage in fruitless discussion as to the nomenclature of such parasites, but rather to consider the flagellate in relation to the life-history of the host. The relation of insect flagellates to their hosts' life-cycle is a most interesting though difficult subject, on which little or nothing has been done in most cases up to the present.

II. THE BIOLOGY OF THE HOST FLIES, *STRATIOMYIA CHAMELEON* AND *S. POTAMIDA*

(*a*) *Habits.* The larvae of these insects are very characteristic, worm-like, legless organisms, armed with powerful jaws by which they drag the body forwards, progression being aided by bristles attached to each segment. The larger larvae examined varied from $\frac{3}{4}$ inch to $2\frac{1}{2}$ inches in length at will, as the abdomen can be retracted telescopically. Their colour varies with that of the mud and decaying vegetation among which they live, yellowish, brown and green being common hues. Twenty-five to thirty respiratory tail filaments are present and are applied by the larva to the surface film, so that in deep water the larva is suspended vertically, head downwards.

The pupae are found near to the surface in the mud fringing the pond or stream. The larval skin is retained and the much smaller pupa is enclosed within it. The period of pupation is short.

The imago is somewhat bee-like and is blackish in colour with bright yellow markings. Eggs are laid on aquatic plants just above the level of the water, and the larvae either escape into the water, or are hatched beneath the surface into which the plants finally fall.

(*b*) *Food.* The food of the imago seems to be sweet vegetable material. The larvae, so far as can be ascertained by direct observations extending over three years, are vegetable feeders. The contents of the alimentary tracts of those examined have never

contained blood or other recognisable animal matter such as muscle or chitinous tissue that could be obtained from insects or Crustacea. Small organisms such as Protozoa may be ingested, but no proof that such was the case occurred. A typical set of examinations of 50 larvae from 10 different localities, representing about one-third of the total number examined, gave the following results:—

From the first locality, seven larvae varying from $\frac{3}{4}$ inch to $2\frac{1}{4}$ in. long, were obtained. Two were infected with Herpetomonas, one infected larva was $1\frac{1}{2}$ in. long, the other $\frac{3}{4}$ in. The large vegetation of the locality comprised grass, *Veronica beccabunga*, *Myosotis palustris*, various algae, and many rotting leaves. The contents of the food canals of the larvae were portions of *Chara*, *Cladophora*, *Spirogyra* and *Zygnema*.

Ten larvae from the second locality were from 1 in. to 2 in. long. None were infected. Grass and *Veronica beccabunga* constituted the larger vegetation. There were fewer large algae than in the first locality. The alimentary canals of these larvae contained algae, chiefly species of *Closterium*, *Penium*, *Micrasterias* and *Cosmarium*.

Six larvae from a third place varied from $1\frac{1}{4}$ in. to $2\frac{1}{2}$ in. long. Like the previous set, they were uninfected. Their gut contents consisted almost entirely of *Gonium* sp., and the larger vegetation on the spot comprised grass, *Myosotis* and Common Hemlock.

A small pond, whose bordering vegetation consisted of grass and *Myosotis*, and in which some fine *Hottonia* grew, yielded seven larvae. These contained many algae, chiefly species of *Scenedesmus* and *Selenastrum*. No parasites were found in the larvae.

Six larvae were obtained from the fifth locality. All were vigorous, and varied from 1 in. to $2\frac{1}{4}$ in. in length. Grass, *Myosotis* and algae were abundant. The food passages of the larvae contained algae, chiefly species of *Navicula*, *Diatoma*, *Synedra*, *Gyrosigma* and *Nitzschia*. No herpetomonads were found.

Some of the smallest larvae were obtained from the sixth locality. They varied from $\frac{1}{2}$ in. to 2 in. in length. Seven were examined. A larva, 1 in. long, had a slight infection with *Herpetomonas stratiomyiae*, the rest were normal. Rotting leaves occurred in the water and hemlock, *Veronica beccabunga* and grass constituted the larger vegetation. Algae, chiefly species of *Navicula*, *Denticula* and *Nitzschia*, together with fragments of leaves, were found in the alimentary canals of the larvae.

Three larvae only were obtained from the seventh locality, whose vegetation was the same as that of the sixth. The gut contents of the larvae also were similar. None were infected.

One specimen, $1\frac{3}{4}$ in. long, obtained from a small brook partly choked with *Potamogeton crispus* and large algae, contained no parasites. Its gut contents included species of *Scenedesmus* and *Cosmarium*, together with fragments of *Chara*.

One larva, 2 in. long, was obtained from a ditch fringed with willowherb. Its gut contained numerous specimens of *Cosmarium* and a very few Herpetomonads also were present.

Two larvae from the tenth locality were uninfected. The chief plants growing in the mud were willowherb and water dropwort, while the food contents of the larvae consisted of species of *Cosmarium* and *Navicula*.

The presence of infected larvae coincided to some extent with the quantity of decaying matter that was present; but sometimes larvae obtained from the foulest sources (not those detailed) were as free from infection as those from relatively uncontaminated places. Larvae were in greatest abundance where much decaying material was present.

Altogether, nearly 150 larvae were examined, and the percentage infected with Herpetomonas was only about 3. The percentage of infection in the pupae examined was somewhat higher.

III. MATERIAL AND METHODS

The larvae, pupae and imagines of *Stratiomyia*, comprising both bred insects and those caught at large, have been used during this investigation. The Herpetomonas has been found most abundantly in the digestive tract of the larva. The alimentary canal has been dissected wherever possible, placed in physiological salt solution and examined in serial teased portions. The pupal condition usually prevented dissection of the alimentary canal, but smears of the central part of the body were sufficient to allow of identification of the parasite. Much time has been spent in observing the living Herpetomonas. Osmic acid followed by absolute alcohol, Bouin's fluid and Flemming's solution have been used for fixatives, and Giemsa's solution, iron haematoxylin and glycerin haematin were used as stains. The paraboloid condenser and stereoscopic oculars have also been used and found of much service in determining depths of granules, chromatin particles and similar structures.

V. THE LIFE HISTORY OF *HERPETOMONAS STRATIOMYIAE*

The life-cycle of the herpetomonad may be divided into two main phases: (1) a resting phase, (2) an active, multiplicative phase. The resting phase again can be sub-divided according to the period at which it is present in the host. Infection is by the contaminative method, and freshly ingested resting forms of the parasite about to develop into the active form are best described as the preflagellate stages, while the forms produced by the preparation of the full flagellate for extra-corporeal life are more accurately described as post-flagellate forms. Naturally the post-flagellate form produced in the first host becomes the pre-flagellate organism in the second host.

A. The Herpetomonad in the Larva of Stratiomyia

The preflagellate stage of *Herpetomonas stratiomyiae* differs from that of most herpetomonads in being elongate oval in shape (Pl. XLI, figs. 1, 2). Its length varies from 5·5μ to 8μ, while

its breadth is about 3μ. The cytoplasm is clear in life or slightly granular. The nucleus shows as an oval, refractile vacuole, the blepharoplast as a bar-like rod. They both lie relatively near the surface. Sometimes the end of the body near the blepharoplast appears somewhat more refractile in life than the distal end. Such an area, when stained, proved to be the chromatophile vacuole-like area from which the flagellum originates. The nucleus presents a definite nuclear membrane, seen best in haematin-stained preparations. The chromatin varies in its arrangement, sometimes appearing as a more or less central concentrated karyosome (fig. 2), at other times being scattered as granules in the nucleoplasm (fig. 1). The bar-like blepharoplast stains deeply. The formation of a flagellum occurs very rapidly, and hence it is difficult to find stages in which the chromatophile area is present unless the host is dissected just at the time preceding the flagellation of the parasites. Division occurs among the preflagellates (figs. 4, 5), and will be described later.

The flagellates (figs. 10-17) vary in size, the length including the flagellum being from 26·6μ to 57μ, while the breadth is from 2μ to 3·6 μ. The flagellum itself may occasionally reach 38μ long. The non-flagellate or posterior end of the flagellate is elongate but somewhat blunted; the flagellar or anterior end is somewhat rounded, with the flagellum projecting from it. As with the preflagellate forms, the nucleus is oval and shows cyclical development. Relatively young flagellates, or flagellates that have formed rapidly from the preflagellate forms, often possess a vesicular nucleus with a central (figs. 15, 16) or slightly excentric (fig. 12) karyosome. Older parasites, those of slow growth and forms about to divide, have their nuclear grains of chromatin evenly distributed (figs. 11, 14). The blepharoplast is curved, rod-like or oval, and, except in dividing forms, is homogeneous in structure. Chromatoid granules may be present in some flagellates, usually forming small grains in the post-nuclear part of the body (figs. 11, 13).

Multiplication. The increase in numbers of the parasites within the host is brought about by longitudinal division, which can take place in either the preflagellate (figs. 3-5) or flagellate (figs. 18-22) stages. The division of the preflagellates is initiated by that of the blepharoplast, which becomes dumb-bell shaped (fig. 3),

showing two concentrations of chromatin, one at each end. The heads of the dumb-bell remain attached to one another for some time by a strand of chromatin. The constriction of the nucleus rapidly follows that of the blepharoplast. The future flagellar end then becomes cleft (fig. 4), and the cleft extends backwards. As soon as parts of the two daughter organisms are free, rapid movements occur, each free part diverging from the other and twisting on itself as it does so, until the two organisms come to lie in almost a straight line (fig. 5). Violent movements precede the final separation, and this is usually succeeded by relative quietness on the part of both the daughter forms, which usually do not move away until some seconds and, occasionally, minutes have elapsed. When movement occurs it is not active as with the flagellates, but consists of slow undulations of the body, which gradually propel the daughter forms forwards.

The stages of division of the flagellate (figs. 18-22) are like those of the preflagellate form, but the division of the blepharoplast and flagellum takes place almost simultaneously (figs. 19, 20), while nuclear division is often delayed, and at times, cleavage of the cytoplasm has commenced (fig. 20) before the nucleus has completely divided. The movements of the two parts of the dividing organism (figs. 21, 22) are very active, and the final separation is effected more quickly than that of the dividing preflagellate, the lashing of the daughter flagella being of material aid. The two newly-formed individuals swim away rapidly after separation. Examination of stained specimens has been confirmatory in all respects of the above observations made on the living organisms.

The formation of a post-flagellate form (figs. 23-36) is brought about by the gradual retraction and absorption of the flagellum (figs. 23-27) and the concentration of the body until it forms an oval or rounded form, which secretes a thin, closely adherent cyst around itself. When the assumption of the post-flagellate form is complete, there is no trace of the flagellum remaining as such. Even its root (or rhizoplast) disappears, the chromatin of it appearing to be dissolved and concentrating, in some cases, in a small part to form a chromatoid area, as well as diffusing generally through the body substance, as shown by the staining reactions of the cyst. The post-flagellate is Leishmania-like (figs. 32-36), of a somewhat

elongate type. The nucleus may, or may not, show a karyosome, for as with the flagellate, the structure of the nucleus varies. If there is a karyosome in being at the time of encystment, the nucleus of the post-flagellate shows the same feature (figs. 34, 36). Should encystment follow rapidly on division, the nucleus is almost homogeneous (figs. 33-35). Much discussion has arisen recently as to the existence of specially differentiated portions of the blepharoplast, described variously as karyosomes and centrioles. In some cases, by the use of iron-haematoxylin, some granules have been demonstrated, but it is admitted that the existence of these granules depends for its demonstration on the degree of removal of the stain by the iron alum. They are not found when other stains are employed. The basing of argument on the fleeting appearances produced by too great or too little differentiation—the degree of differentiation that is 'accurate' being an absolutely individual opinion—is merely vexatious, and does not tend to the advancement of knowledge on really scientific lines.

B. The Herpetomonad during Pupation

As the period for pupation of the host approaches, the post-flagellate forms of the parasite leave the gut of the larval host and pass out with the faeces. Rapid flagellation of any preflagellates that are present occurs, together with division of the flagellates. The organisms seem to collect in the middle-third of the gut and place themselves with their flagella towards the centre of its lumen, their aflagellar ends being in contact with its wall. The latter is very frail at this time, and the flagellates pass through it with ease. They thus reach the haemocoel, and there they swim for a short time in their normal position with their flagella forwardly directed. Division occurs but infrequently. The flagellates (figs. 23-25) gradually slow their movements and then rapidly concentrate their substance (fig. 27). They soon become typical, post-flagellate forms (figs. 27-31), with distinct nuclei and blepharoplasts, and very thin cyst walls. These organisms are, perhaps, somewhat more frail-looking than those found in the rectum and faeces of the larva (figs. 32-36). At first they are intermingled with the numerous wander cells found in the pupae at this time, but they seem to prefer the more fluid parts of the host. The result is that, in a late pupa

in which the musculature has become fairly well developed, the post-flagellates lie in the more fluid medium, internal to the region of differentiated muscles. At times, they have been found crowded together roughly at the centre of the pupa, and seem to be united temporarily by gelatinous material. When the differentiation of the alimentary canal is in progress, the parasites in most cases become surrounded by it and can be found attached by the gelatinous secretion to the cells of the gut. They remain thus until the emergence of the imago. Four out of fifteen pupae were found infected, but in one only was the infection heavy.

C. The Herpetomonad in the Imago

The number of infected imagines is much smaller than that of infected larvae, though neither are abundant. The occurrence of larvae in numbers in one district enables them to become infected with post-flagellates from their neighbours with a certain amount of ease, as they swallow the cysts with their food. But all infected larvae do not give rise to infected pupae, while the latter may have but a slight infection that fades out and disappears in the adult. The habits of the imagines do not readily allow of them acquiring infection *ab initio*, and consequently the numbers found infected have been extremely small. The parasites have been found in all stages. Non-flagellates, like those in the pupa, have been found in the gut near the junction of the thorax and abdomen. They rapidly form flagellates that spread quickly throughout the whole length of the gut, while post-flagellates occur in the rectum and faeces. No stages of the parasite have been observed up to the present in the genital organs and but few in the haemocoel of the host. When there is haemocoelic infection, there are two possible means of origin: (1) the parasites may have remained there from the pupation period, or (2) they may have penetrated from the gut as flagellates. As they are capable of moving and developing fully in the haemocoelic fluid, hereditary infection is possible, though we are not in a position to pronounce on this with certainty, owing to the small number of infected flies that have been examined and to the difficulty of obtaining eggs. We hope to continue this part of the work in the future.

V. MODE OF INFECTION

The mode of infection is contaminative originally. The larvae acquire post-flagellate forms of the Herpetomonas with their food. The cysts may have been derived either from already infected larvae or from the dejecta of adult flies. The faeces of a larva on one occasion contained active flagellates as well as post-flagellate forms. As the flagellates lived for some hours in ordinary water, it is possible that they could do so in nature, and act as an additional source of infection of new hosts.

The parasite can persist through the metamorphosis of the host and the imagines thus emerge infected. It is possible, though we think it is probably exceptional, that an adult insect may become infected by sucking plant juices contaminated with excrement from other imagines, and thus acquiring the post-flagellate stages of the parasite.

VI. CONCLUDING REMARKS

The flagellate parasite of *Stratiomyia chameleon* and *S. potamida* is a member of the genus Herpetomonas as originally defined. It differs from *H. pediculi* (Fantham, 1912) from the body louse. *Pediculus vestimenti*, in its characteristic, oval, preflagellate form. *Herpetomonas jaculum* from *Nepa cinerea* is similar to *H. stratiomyiae* in appearance, but again the preflagellates of *H. stratiomyiae* are distinctive, the same feature differentiating the parasite from *H. lygaei* and *H. culicis*. It also shows differences from *H. muscae domesticae*, *H. ctenocephali*, *H. ctenophthalmi* and other known Herpetomonads. In consequence of these morphological differences, and as it is the first time that such a parasite has been recorded from the Stratiomyidae, the organism has been named *Herpetomonas stratiomyiae*. We believe that this account is the first detailed record of the behaviour of an insect flagellate during the pupation of its host, a feature which gives a special interest to *Herpetomonas stratiomyiae*.

The study of the life-cycle of such herpetomonad flagellates of insects is of the utmost importance in view of the recent experiments of Laveran and Franchini (1913) on the successful experimental inoculation of *H. ctenocephali* (Fantham, 1912), from the gut of the dog-flea, into mice and other mammals. Such researches suggest an experimental leishmaniasis in the making.

VII. SUMMARY

1. *Herpetomonas stratiomyiae*, n. sp., is a parasite of the larvae, pupae and imagines of the flies, *Stratiomyia chameleon* and *S. potamida*.

2. The herpetomonads present three stages in their life-history, (*a*) a preflagellate stage, (*b*) a flagellate stage and (*c*) a post-flagellate stage.

3. Preflagellate forms are characteristically oval, with elongate nucleus and distinct blepharoplast. The flagellates vary from 26·6μ to 57μ in total length. The post-flagellates, as found in the hind gut of the larvae, are oval, with somewhat thicker walls than the preflagellates.

4. Multiplication is by longitudinal division and can occur in any phase of the life-history of the organism.

5. Just prior to pupation, the flagellates migrate from the gut of the larva into the haemocoel where they gradually become motionless, withdraw their flagella and assume the post-flagellate form. They collect in the more fluid parts of the body of the pupa, and, ultimately, as a result of this, they become enclosed in the gut of the adult.

6. The imago, if formed from an infected pupa, usually emerges from the pupa case infected with non-flagellate forms of the herpetomonad. The development of the non-flagellate into the flagellate form is rapid, and soon all stages of the organism are present.

7. Infection of the larvae is contaminative, that of the pupa is transmitted from the larva, while the imago may retain the pupal infection or may possibly acquire it *ab initio* by ingesting contaminated food.

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EXPLANATION OF PLATE XLI

All the figures were outlined with an Abbé-Zeiss camera lucida, using one-twelfth inch oil immersion lens and compensating ocular No. 8. Magnification 1,500 diameters, approximately.

Figs. 1-22. Illustrate the preflagellate and flagellate stages found in the larva.

Figs. 1-9. Illustrate the development of *Herpetomonas stratiomyiae* from the preflagellate to the flagellate stage.

Fig. 1. Preflagellate form with nuclear chromatin evenly distributed.

Fig. 2. Preflagellate with nucleus showing a karyosome.

Figs. 3-5. Stages in the division of a preflagellate.

Fig. 3. Preflagellate with blepharoplast dividing.

Fig. 4. Form with blepharoplast and nucleus dividing.

Fig. 5. Separation of the daughter organisms.

Figs. 6-9. Show the development of a flagellum and assumption of the flagellate form.

Figs. 10-16. A series of flagellates showing variation in size and appearance.

Figs. 11, 13. Show flagellates containing chromatoid granules.

Fig. 17. An aggregation rosette of flagellates of different ages, attached by their flagella to a piece of débris.

Figs. 18-22. Stages in the division of flagellates.

Fig. 18. Parasite showing constriction of blepharoplast.

Fig. 19. Parasite with flagellum splitting.

Fig. 20. Form with flagella and blepharoplast separate and nucleus dividing.

Fig. 21. Commencement of division of the general body cytoplasm.

Fig. 22. Daughter organisms almost separate.

Figs. 23-36. Illustrate post-flagellate stages obtained from larvae and pupae.

Figs. 23-25. Transitional forms between flagellate and post-flagellate stages, as obtained from the haemocoel of the larva.

Fig. 26. Similar transitional flagellate from the hind gut of a larva.

Figs. 27-31. Post-flagellates from pupae.

Figs. 32-36. Post-flagellates from the hind gut of a larva.

THE CULTURE OF *BABESIA (PIROPLASMA) CANIS* IN VITRO

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Plate XLII

CONTENTS

	PAGE
Introduction	621
Technique	622
Progress of the Cultures	622
Morphology of the Cultural Forms	624
Division of Parasites in Vitro	626
Successful Inoculation of the Animal Host from a 41 hours' Culture	629
Summary	629
References	630
Explanation of Plate	632

INTRODUCTION

The present paper contains the preliminary results of the authors' investigations on the cultural forms of *Babesia canis*. The importance of the investigation of cultural forms of Protozoa does not need emphasis, and many practical applications are suggested, but in this paper we confine ourselves to some remarks on the morphology and life-history of the Sporozoön as seen in the culture tube. The method of C. C. Bass (1912) has been followed, without any addition or modification, though the parasite is not so easily cultivated as the Plasmodia of man.

TECHNIQUE

Cultivation succeeded in two out of four attempts. In each successful case the infected animal, whose blood was used, was a puppy about three months old. The blood used in the first culture was taken from the heart on the fifth day after inoculation, in the second case on the fourth day after inoculation.

Ten c.c. of heart blood, drawn with aseptic precautions, was mixed with 1/10 c.c. of a 50 per cent. aqueous solution of Merck's glucose. The blood was carefully and gently defibrinated by means of a rod, and the clot was removed. It was noticed that the amount of clot removed was much in excess of that taken from a similar quantity of human malarial blood. The defibrinated blood was distributed into smaller tubes, placing about one inch of liquid in each tube. No centrifugalisation was necessary. The tubes were incubated at 37° C. The corpuscles settled to the bottom in a short time, leaving a layer of serum above.

Marked haemolysis was seen in all the cultures attempted. It was found advantageous to take the blood of the puppy before the crisis, that is, before too many parasites were present in the peripheral or heart blood.

No sodium citrate nor ascitic fluid was added to the cultures, as was done by Ziemann (1913), nor citrate and saline as used by Toyoda (1913).

PROGRESS OF THE CULTURES

We will set forth in some detail the progress of events in our FIRST CULTURE, which was more successful than the second, in that more divisions occurred in it.

In the original heart blood, before inoculation, the parasites were not very numerous, pairs or singles being found in the infected red blood corpuscle (Pl. XLII, fig. 1). Only one group of four parasites was seen in any corpuscle.* The Babesia were chiefly pyriform in shape, with small compact nuclei, and the secondary

* Graham-Smith (1905) found that the infected red blood corpuscles containing more than four *Babesia canis* constituted less than 0·3 % of the total. He counted 22,589 infected corpuscles from peripheral and heart blood of dogs. Corpuscles containing one and two parasites formed 96·4 % of the total. (*Journ. Hygiene*, V, p. 252)

loose mass of chromatin was not well marked. Phagocytosis was evident, one large mononuclear leucocyte had ingested four infected corpuscles (Pl. XLII, fig. 2).

After 7 *hours* large numbers of intracorpuscular parasites occurred in clumps, especially at the margins of the smears. The infected corpuscles usually contained four parasites each (Pl. XLII, fig. 3), a few contained eight merozoites (Pl. XLII, fig. 4). Hence the original parasites had divided once. The piroplasms, usually pyriform, now showed clearly the secondary loose mass of chromatin. Examples of division by budding and chromatin forking (Pl. XLII, fig. 5), as described by Nuttall and Graham-Smith (1907) from dog's blood, were seen.

After 15 *hours* numerous infected corpuscles contained four parasites, some corpuscles contained eight, while a very few showed sixteen merozoites (Pl. XLII, fig. 7).

At 24 *hours* there was an increase in the number of infected corpuscles containing eight parasites, as well as in those containing sixteen (Pl. XLII, fig. 8). Corpuscles containing four piroplasms were also present. Rounded dividing forms, exhibiting chromatinic forking were found. There was thus evidence of another division beginning.

After 30 *hours* some parasites were seen to be degenerating. Corpuscles containing eight living piroplasms were numerous. The parasites seemed to have grown larger.

After 48 *hours* numerous corpuscles were found to contain sixteen merozoites (Pl. XLII, fig. 9). One cluster of 29 was found. Three of the parasites in this group each showed two chromatinic dots, and were probably about to divide, so that the cluster represented 32 daughter forms derived from one parent Babesia. The host corpuscle had burst, but its remains could be distinguished.

At 60 *hours* the parasites were few and were smaller in size. Two groups of eight and one of four were noticed in one smear. These parasites had circular chromatin masses. No others were seen on this smear. Most of the cultural piroplasms were now dead, and at 68 hours none was found.

Three divisions had occurred in the cultures, from twos, through fours to eights and sixteens in infected red blood corpuscles.

Heart blood of the dog, kept as control, and incubated at 37° C.,

showed a few somewhat shrunken parasites after 24 hours, and no parasites after.

In our SECOND CULTURE only pairs or single pyriform parasites were found in the heart blood before incubation. This culture progressed more slowly than the former.

After 6 *hours'* incubation there was more variety in the form of the parasites, some being larger and round and some amoeboid.

At 7 *hours* a very few parasites were showing the commencement of gemmation.

After $8\frac{1}{2}$ *hours* some free pyriforms were found and an intracorpuscular group of four. The nuclei of these contained small dots (karyosomes) and a little loose chromatin.

At 16 *hours* fours were more numerous, and distinct chromatinic budding was seen in several specimens.

After $18\frac{1}{2}$ *hours* there were numerous groups of four pyriform parasites, a few eights and one group of twelve. The loose chromatin was well marked. There was evidence of various types of division. Some degenerating forms were now noticed.

At $23\frac{1}{2}$ *hours* corpuscles were seen containing one, two and four parasites.

At $24\frac{1}{2}$ *hours* groups of four merozoites were fairly common.

At 30 *hours* many fours were present and some groups of eight.

At 41 *hours* groups of fours were still found, and $\frac{1}{2}$ c.c. of the culture, containing most of the corpuscles, was inoculated into a young puppy. The puppy developed piroplasmosis and succumbed five days later. The remains of the culture were examined at about 66 hours and no live parasites were seen.

There is thus evidence that only two divisions occurred in this culture. It certainly did not grow so well nor so rapidly as the first. We are unable to explain the cause of this difference in the progress of the two cultures, but we may remark that the strain in the second case was of a more chronic character.

MORPHOLOGY OF THE CULTURAL FORMS

The piroplasms were examined fresh, and after fixation and staining. Smears were made from time to time and fixed by the wet and by the dry methods, and stained by Romanowsky, Giemsa

and haematin stains. Bouin's fluid was used in some cases as a fixative.

In this paper we do not propose to deal exhaustively with the morphology of the cultural forms of *Babesia canis*, but to record only the more important findings.

The parasites exhibited marked variation in shape. Pyriform, amoeboid, round and oval types were seen (Fig. 1, A—D). The method of gemmation and chromatinic forking was observed, as first described by Nuttall and Graham-Smith from the blood of the dog. There was also evidence of binary fission.

The pyriform piroplasms (Fig. 1, A—B) usually exhibited a distinct nucleus, as a dot of chromatin, often surrounded by a clear achromatic halo. Such a nucleus is of the karyosomatic type, the chromatinic dot representing the karyosome. Such a structure has

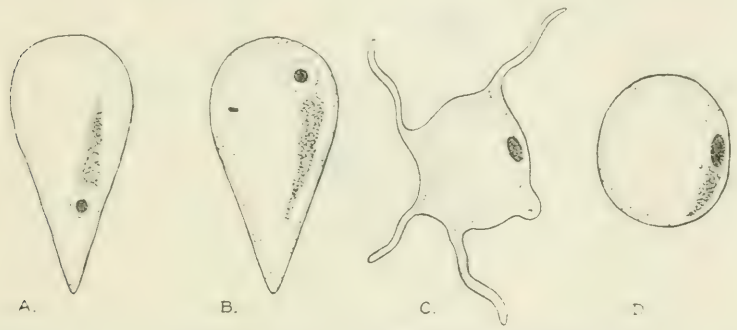

Fig. 1. Various forms of *Babesia canis* in culture.
A, B. Two pyriform parasites showing variation in nuclear position. Loose chromatin also present.
C. Amoeboid form. D. Rounded form.

been recorded in *Babesia canis* by Schuberg and Reichenow (1912). The presence of a very thin nuclear membrane is sometimes suggested, but at other times such a nuclear membrane is certainly not well marked. A secondary mass of loose chromatin, of a reticulate or 'woolly' character is also seen, as described from blood by Nuttall and Graham-Smith and by Christophers (1907) in *Babesia canis*, and by one of us (Fantham, 1907) in *Babesia bovis*. The secondary mass of chromatin was well seen in 7-hour cultures. In some pyriform parasites a very small dot of chromatin was observed (Fig. 1, B), the so-called blepharoplast of Schaudinn and Lühe;

but this punctiform chromatinic mass is not comparable with the blepharoplast of a flagellate.

Pyriform Babesia in cultures of 7 hours' duration had the nucleus usually near the pointed end (Fig. 1, A), while after 24 hours' culture it was sometimes seen to be near the rounded or blunt end of the parasite (Fig. 1, B). Thus the position of the nucleus may vary in different specimens.

Some amoeboid forms with fine pseudopodia were present, especially in 7 hour cultures (Fig. 1, C). Such parasites were also found, motile, on examining fresh preparations.

Rounded forms often showed signs of division by the method of gemmation.

FIG. 2. Infected corpuscle containing four parasites. The upper one of the four is oval, and is Leishmania-like; the lower three show masses of loose chromatin.

In one case, in a 15 hours' culture, a remarkable Leishmania-like oval form was seen, with nucleus and blepharoplast (Fig. 2, oval parasite).

DIVISION OF PARASITES IN VITRO

Many examples were found of the mode of gemmation with chromatinic forking (Figs. 3, 4), now made a diagnostic character of the genus Babesia. Round piroplasms protrude two small buds symmetrically arranged to one side (Fig. 3, C), which buds contain chromatinic cores connected with the main nucleus (Fig. 3,

D—G). In some cases the buds are almost entirely composed of chromatin at first, and seem as if they are about to separate from the parent. The buds then grow at the expense of the rounded

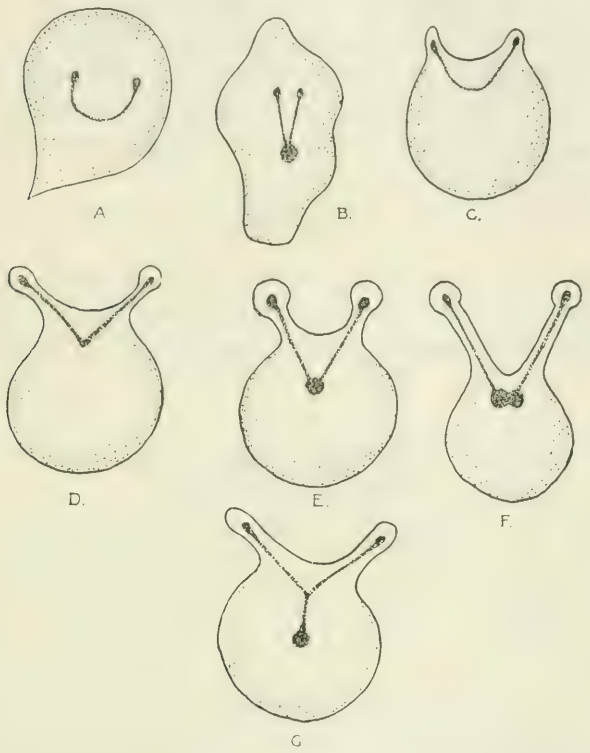

Fig. 3. Babesia showing division by gemmation, accompanied by chromatin budding and forking.

A. Parasite with arcuate chromatin.

B. Somewhat amoeboid parasite with chromatin fork.

C. Shows small, symmetrical cytoplasmic buds and chromatin processes entering buds.

D. Parasite with larger buds; main chromatin not distinguishable, but chromatin cores well marked.

E. Larger buds shown. Well marked main chromatin mass and processes.

F. Parasite showing commencement of fission of main chromatin mass.

G. Form showing typical Y shaped chromatin bifurcation.

portion of the parent parasite, and the linear processes of chromatin give rise to the loose mass of secondary chromatin seen in the daughter pyriform piroplasms. Early stages of chromatinic

budding and forking were seen while the parasite was still round or somewhat amoeboid (Fig. 3, B) in contour. Various stages of division may be observed concurrently in several parasites in one corpuscle (Fig. 4). Certain cases were noticed in which the chromatinic forking did not assume the typical Y form (Fig. 3, G), but was arcuate in character (Fig. 3, A). Sometimes it is difficult (*vide* Fig. 3, D) to observe the main chromatin mass from which the forks arise. It is unnecessary to enter into a lengthy description of this mode of division, as it has already been described by various

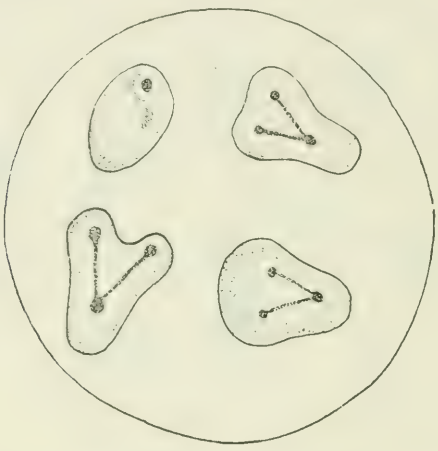

Fig. 4. Infected corpuscle showing parasites in various stages of division.

authors from the blood of dogs. The present is the first record of its occurrence in cultures, and we also succeeded in seeing the method in operation in living parasites taken from cultures.

Christophers (1907) considers that, beside the mode described above, there is also division by direct binary fission. Nuttall and Graham-Smith have recorded such direct division in the case of rounded blood forms of *Babesia canis*. We agree with the above-mentioned workers, as parasites of various shapes are often seen with two and even four principal chromatinic masses (Fig. 5, A—B). We have also seen parasites whose nucleus exhibited a form of promitosis. Further, we have observed long, somewhat sausage-shaped parasites, containing two chromatin masses, and others

indented in the middle, apparently dividing into two pyriforms, as figured by Christophers (1907, p. 21).

Fig. 5. Two parasites showing direct division. A has two nuclei, B has four chromatin masses.

The free forms of the piroplasms, seen in cultures, which were attempting to enter fresh blood corpuscles, were invariably of the pyriform type, and were endeavouring to enter by the blunt or rounded ends.

SUCCESSFUL INOCULATION OF THE ANIMAL HOST FROM A 41 HOURS' CULTURE

A young puppy was inoculated successfully with about $\frac{1}{2}$ c.c. of a 41 hours' culture of *Babesia canis*. The inoculation was performed intraperitoneally. The puppy showed numerous parasites on the early morning of the fifth day after inoculation, and died on the evening of the same day.

Unfortunately, the removal of a series of portions of the culture for examination, has hitherto left us with insufficient material to attempt sub-cultures. We have, however, no doubt that such sub-cultures would be successful, as was shown by Ziemann (1913).

SUMMARY

1. We have succeeded in cultivating *Babesia* (*Piroplasma*) *canis* in two out of four attempts, following the method of Bass, using blood and glucose, and incubating at 37° C.

2. In one of these cultures, starting with heart blood containing corpuscles infected with one, two or, exceptionally, four piroplasmata, we succeeded in obtaining a maximum of 32 merozoites in a corpuscle.

3. Various types of Babesia were seen in these cultures, namely, pyriform, amoeboid, rounded and oval parasites. Division of rounded forms was observed, following the method of gemmation with chromatinic forking. There was evidence, in stained specimens, of direct binary fission.

4. Haemolysis occurred in all the culture tubes.

5. A puppy was successfully inoculated from a 41 hours' culture and succumbed to piroplasmosis on the fifth day.

6. *Babesia canis* is not so easily cultivated by Bass's method as the malarial parasites of man.

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EXPLANATION OF PLATE XLII

Fig. 1. Microphotograph showing pyriform *Babesia canis* from heart blood of puppy before incubation.

Fig. 2. Shows phagocytosis of four infected red blood corpuscles. From heart blood before incubation.

Fig. 3. Microphotograph showing infected corpuscles containing two and four Babesia. Seven hours' culture.

Fig. 4. Division rosette of eight merozoites from seven hours' culture. Some show loose chromatin.

Fig. 5. Two parasites in process of division by the method of gemmation and chromatin forking. Seven hours' culture.

Fig. 6. Shows mononuclear leucocytosis in piroplasmosis, together with groups of parasites, from a 15 hours' culture.

Fig. 7. Groups of eight and four parasites from a 15 hours' culture.

Fig. 8. Group of sixteen merozoites from a 24 hours' culture.

Fig. 9. Group of sixteen merozoites from a 48 hours' culture, showing increase in size.

INDEX

INDEX

PAGE

INDEX OF AUTHORS iii

GENERAL INDEX iii

INDEX OF SPECIES NEW TO SCIENCE viii

INDEX OF AUTHORS

PAGE

Balfour, A. 113
Barratt, J. O. Wakelin 367
Blacklock, B. 101
Blacklock, B.; and Stephens, J. W. W. 303
Blacklock, B.; and Yorke, W. ...563, 603
Carter, H. F. 581
Chalmers, A. J.; and O'Farrell, W. R. 525
Chalmers, A. J.; and Stirling, A. D. ... 541
Christophers, S. R. 45
Darling, S. T. 321
Fantham, H. B.; and Porter, A. 569, 609
Fantham, H. B.; and Stephens, J. W. W. 27
Fantham, H. B.; and Thomson, J. G. 621
Fantham, H. B.; Thomson, J. G.; and Thomson, D. 153
Kinghorn, A.; Yorke, W.; and Lloyd, Ll. 183
Lloyd, Ll.; Kinghorn, A.; and Yorke, W. 183
Lloyd, Ll.; and Wallace, A. F. 299
Macfie, J. W. Scott. 1, 339, 359
Marsden, P. H. 335
Mayer, T. F. G. 41
Newstead, R. 331
O'Farrell, W. R. 545
O'Farrell, W. R.; and Chalmers, A. J. 525

PAGE

Porter, A.; and Fantham, H. B. 569, 609
Rogers, W. 363
Ross, G. A. Park 371
Scott, H. Harold 165
Seidelin, Harald 377
Stephens, J. W. W. 479
Stephens, J. W. W.; and Blacklock, B. 303
Stephens, J. W. W.; and Fantham, H. B. 27
Stirling, A. D.; and Chalmers, A. J. 541
Theobald, Fred. V. 591
Thomson, David 125
Thomson, D.; Fantham, H. B.; and Thomson, J. G. 153
Thomson, D.; and Thomson, J. G. ... 509
Thomson, J. G.; and Fantham, H. B. 621
Thomson, J. G.; and Thomson, D. ... 509
Thomson, J. G.; Thomson, D.; and Fantham, H. B. 153
Todd, John L. 309
Wallace, A. F. 301
Wallace, A. F.; and Lloyd, Ll. 299
Yorke, W.; and Blacklock, B. ...563, 603
Yorke, W.; Lloyd, Ll.; and Kinghorn, A. 183

GENERAL INDEX

PAGE

Aedimorphus quinquepunctata, nov. sp. 598
Ankylostomiasis, prevention in the Panama Canal Zone 137
Anophelinae, classification and phylogeny of 81
„ Colour marking other variable characters of 45
„ Geographical distribution of 84
„ Grouping of species ... 87

PAGE

Babesia canis, cultivation in vitro ... 621
„ „ morphology in cultures 624
Balfour, A. Trypanosomiasis in the Sudan and notes on tsetse fly traps and immune breed of cattle. 113
Banksinella, Theobald, mosquitos of the genus 581
Barratt, J. O. W. Recent experimental research bearing upon blackwater fever 367

PAGE

Blacklock, B. Posterior nuclear forms of *T. rhodesiense* in rats 101
Blacklock, B., and Stephens, J. W. W. Non-identity of *T. brucei* with the trypanosome of the same name from the Uganda ox (*T. ugandae*, sp. nov.) 303
Blacklock, B., and Yorke, W. *Trypanosoma vivax* in rabbits 563
Blacklock, B., and Yorke, W. The probable identity of *T. congolense* (Broden) and *T. nanum* (Laveran) 603
Blackwater fever, Correlation between malaria and blackwater statistics... 499
,, ,, Effect of period of residence 488
,, ,, Influence of malaria 486
,, ,, Malarial parasites ... 479
,, ,, Pigmented leucocytes 485
,, ,, Post-mortem examinations 485
,, ,, Recent experimental research bearing upon 367
,, ,, Relationship to species of malarial parasite 486
,, ,, Seasonal prevalence 492
,, ,, Second attacks 504
,, ,, Statistical 479
British Guiana, Sanitation in 143
Carter, H. F. Mosquitos of the genera *Banksinella*, Theobald, and *Taeniorhynchus*, Arribalzaga 581
Cattle breed immune to trypanosomiasis 119, 124
Cerebro-spinal meningitis, fulminating, in Jamaica 165
Chalmers, A. J., and O'Farrell, W. R. The Trichonocardiases 525
Chalmers, A. J., and Stirling, A. D. Epidemic Trichonocardiasis 541
Christophers, S. R. Colour marking and other variable characters of *Anophelinae* 45
Chrysoconops nocturnus, nov. sp. 593
Crithidia hyalommae, hereditary infection with, of *Hyalomma aegyptium* ... 545

PAGE

Crithidia hyalommae, Morphology ... 549
Culicelsa centropunctata, nov. sp..... 599
Culicidae from the Sudan 591
Darling, S. T. Identification of the pathogenic entamoeba of Panama... 321
Diplococcus jamaicensis, sp. nov. 465
,, ,, Causing vomiting sickness in Jamaica ... 465
Domestic Animals, Trypanosomiasis in 1
Dysentery, prevention in the Panama Canal Zone 138
Entamoeba, feeding and inoculation experiments 326
,, Identification of the pathogenic, of Panama 321
Fantham, H. B., and Porter, A. The pathogenicity of *Nosema apis* to insects other than hive bees..... 569
Fantham, H. B., and Porter, A. *Herpetomonas stratiomyiae*, n.sp., a flagellate parasite of the flies, *Stratiomyia chameleon* and *S. potamida*, with remarks on the biology of the hosts 609
Fantham, H. B., and Stephens, J. W. W. Measurements of *T. rhodesiense* and *T. gambiense* 27
Fantham, H. B., and Thomson, J. G. The culture of *Babesia* (*Piroplasma*) *canis* in vitro 621
Fantham, H. B., Thomson, J. G., and Thomson, D. Cultivation of *Plasmodium vivax* in vitro..... 153
Filaria loa, note on a case 363
Fulminating cerebro-spinal meningitis in Jamaica 165
Glossina in N. Nigeria 7
,, *austeni* in German East Africa 331
,, *morsitans*, breeding places of 289
,, ,, Development of *T. rhodesiense* in ... 273
,, ,, Experimental transmission of *T. rhodesiense* ... 187
,, ,, pupation period influenced by temperature ... 287
,, ,, *T. rhodesiense* in 215
,, ,, Trypanosomes in wild 239

PAGE

Glossina severini, sp. n. 331
" " in the Congo Free State 331
Heptaphlebomyia kingii, nov.sp..... 601
Hereditary infection, with special reference to its occurrence in *Hyalomma aegyptium* infected with *Crithidia hyalommae* 545
Herpetomonas stratiomyiae, nov. sp.... 609
" " in larva of *Stratiomyia* 612
" " in pupa of *Stratiomyia* 615
" " in imago of *Stratiomyia* 616
" " mode of infection... 617
Hyalomma aegyptium, hereditary infection with *Crithidia hyalommae*... 545
Ipomoea, root of, from Rhodesia 335
Isigwebedhla, a fictitious native disease 371
Jamaica, Fulminating cerebro-spinal meningitis in 165
" *Stegomyia fasciata* in 467
" Vomiting sickness in 377
" Yellow fever in 467
Kinghorn, A., Yorke, W., and Lloyd, Ll. Final report, Luangwa Sleeping Sickness Commission 183
Kingia maculoabdominalis, nov. sp. ... 597
Lloyd, Ll., Kinghorn, A., and Yorke, W. Final report, Luangwa Sleeping Sickness Commission 183
Lloyd, Ll., and Wallace, A. F. Experiment to ascertain whether Tabanids transmit trypanosomes in nature 299
Loa loa, A case of 363
Luangwa Sleeping Sickness Commission, final report 183
Macfie, J. W. S. Trypanosomiasis of domestic animals in N. Nigeria..... 1
Macfie, J. W. S. Morphology of the trypanosome (*T. nigeriense*, n.sp.) from a case of sleeping sickness from Eket, S. Nigeria 339
Macfie, J. W. S. The development of a human trypanosome in the gut of *Stomoxys nigra* 359
Malaria, prevention in the Panama Canal Zone 130

PAGE

Malarial Parasites, Cultivation of..... 510
" " Cultivation of *P. vivax* in vitro... 153
" " Growth and sporulation in the culture tube and in the human host 509
Marsden, P. H. Root of an *Ipomoea* from Rhodesia 335
Mayer, T. F. G. Mosquito-proof and storm-proof house 41
Meningitis, Fulminating cerebro-spinal, in Jamaica 165
Micrococcus castellanii, sp. nov..... 531
" " In trichonocardiasis ... 528
" *nigrescens*, in trichonocardiasis 528
Mosquito-proof and storm-proof house for tropics 41
Mosquitos of the genera *Banksinella* and *Taeniorhynchus*..... 581
Mucidus nigerrimus, nov. sp..... 591
Muscidae in N. Rhodesia 296
Newstead, R. New tsetse-fly from the Congo Free State; and the occurrence of *G. austeni* in German East Africa 331
Nocardia tenuis in trichonocardiasis... 528
N. Nigeria, *Glossina* in 7
" Trypanosomiasis in domestic animals in 1
Nosema apis, pathogenicity to insects other than hive bees 569
" " in various Hymenoptera 570
" " in various Lepidoptera 572
" " in various Diptera 575
O'Farrell, W. R. Hereditary infection, with special reference to its occurrence in *Hyalomma aegyptium* infected with *Crithidia hyalommae* 545
O'Farrell, W. R., and Chalmers, A. J. The Trichonocardiases 525
Ornithodorus moubata in N. Rhodesia 293
" " transmission experiment with *T. rhodesiense* 301
Panama Canal Zone, Sanitation in ... 125
" Pathogenic Entamoeba of ... 321
Piroplasma canis, cultivation of..... 621

PAGE

Plague, prevention in the Panama Canal Zone 137
Plasmodium falciparum, Morphology in culture..... 513
„ „ Morphology in the human host 516
„ *vivax*, Cultivation in vitro 153
„ „ Morphology in culture 518
„ „ Morphology in the human host ... 519
Porter, A., and Fantham, H. B. The pathogenicity of *Nosema apis* to insects other than hive bees..... 569
Porter, A., and Fantham, H. B. *Herpetomonas stratiomyiae*, n.sp., a flagellate parasite of the flies, *S. chameleon* and *S. potamida*, with remarks on the biology of the hosts 609
Pupipara in N. Rhodesia 297
Reedomyia sudanensis, nov. sp..... 595
Rhodesia, Northern, blood-sucking insects and ticks in the Luangwa Valley 293
„ „ Meteorological observations 189, 190
Rogers, W. A note on a case of *Loa loa*..... 363
Ross, G. A. P. A fictitious native disease (*Isigwebedhla*) 371
Sanitation in the Panama Canal Zone 125
Scott, H. H. Fulminating cerebro-spinal meningitis in Jamaica..... 165
Seidelin, H. 'Vomiting Sickness' in Jamaica 377
Sleeping Sickness Commission, Final report of the Luangwa 183
„ „ Distribution of, due to *T. rhodesiense* 185
„ „ in Eket, S. Nigeria 339
„ „ symptomatology ... 186
„ „ Sex and age of Africans suffering from 309
Southern Nigeria, sleeping sickness due to *T. nigeriense*, n.sp..... 339

PAGE

Sudan, cattle breed immune to trypanosomiasis 119, 124
„ New Culicidae from the 591
„ Trypanosomiasis in 113
Stegomyia fasciata in Jamaica 467
Stephens, J. W. W. Studies in blackwater fever 479
Stephens, J. W. W., and Blacklock, B. Non-identity of *T. brucei* with the trypanosome of the same name from the Uganda ox (*T. ugandae*, sp. nov.) 303
Stephens, J. W. W., and Fantham, H. B. Measurements of *T. rhodesiense* and *T. gambiense* 27
Stirling, A. D., and Chalmers, A. J. Epidemic Trichonocardiasis 541
Stomoxys possibly transmitting trypanosomiasis 8
„ *nigra*, trypanosome in gut of 359
Stratiomyia chameleon, infected with *Herpetomonas stratiomyiae* 609
„ „ Biology of 610
„ *potamida*, infected with *Herpetomonas stratiomyiae* 609
„ „ Biology of 610
Stratiomys, *vide Stratiomyia* 609
Tabanidae in N. Rhodesia 293
Tabanus, trypanosome transmission experiment 299
Taeniorhynchus, Theobald, mosquitos of the genus..... 585
Theobald, F. V. New Culicidae from the Sudan 591
Thomson, D. Sanitation in the Panama Canal Zone, Trinidad and British Guiana..... 125
Thomson, D., Fantham, H. B., and Thomson, J. G. Cultivation of *Plasmodium vivax* in vitro..... 153
Thomson, D., and Thomson, J. G. Growth and sporulation of the benign and malignant tertian malarial parasites in the culture tube and in the human host..... 509
Thomson, J. G., and Fantham, H. B. The culture of *Babesia* (*Piroplasma*) *canis* in vitro..... 621

PAGE

Thomson, J. G., and Thomson, D. Growth and sporulation of the benign and malignant tertian malarial parasites in the culture tube and in the human host..... 509
Thomson, J. G., Thomson, D., and Fantham, H. B. Cultivation of *Plasmodium vivax* in vitro..... 153
Todd, J. L. Sex and age of Africans suffering from Trypanosomiasis..... 309
Trichonocardiasis 525
,, epidemic 541
Trinidad, Sanitation in 141
Trypanosomes in the gut of *Stomoxys nigra* 359
,, in wild *G. morsitans* ... 239
,, of game and domestic stock 227
,, Transmission experiment with Tabanids 299
Trypanosomiasis, Cattle breed immune to..... 119, 124
,, Human, *vide* Sleeping Sickness.
,, Immunity 10
,, In cattle, seasonal distribution 7, 8
,, Incubation and duration of, due to *T. rhodesiense* 103
,, In the Lado(Western Mongalla, Sudan) 113
,, Of domestic animals in N. Nigeria 1
,, Prophylaxis 9
,, Transmitted possibly by *Stomoxys* 8
,, Treatment 9
,, Tsetse fly traps 118, 124
T. brucei in domestic animals 2, 3, 4
,, Non-identity with the trypanosome of the same name from the Uganda ox (*T. ugandae*, sp. nov.) 303
,, Posterior nuclear forms..... 3
T. congolense from donkey 122
,, Probable identity of, and *T. nanum* 603
T. gambiense, Measurements of 27
T. ignotum, sp. nov..... 263
,, Diagnosis 266

PAGE

T. ignotum In wild *G. morsitans*, 240, 242
,, Measurements 264
,, Morphology 263
,, Pathogenicity 265
,, Transmission 269
T. multiforme, sp. nov..... 254
,, Diagnosis 257
,, In game in N. Rhodesia 233
,, Measurements 255, 259
,, Morphology 254
,, Pathogenicity 257
,, Transmission 261
T. nanum In domestic animals, 2, 3, 7, 235
,, In game in N. Rhodesia, 231, 233, 234
,, Measurements 249
,, Morphology 248
,, Pathogenicity 248
,, Probable identity of, and *T. congolense* 603
,, Transmission 251
T. nigeriense, n. sp. 356
,, Animal reactions 345
,, Measurements 348
,, Morphology 343
T. pecorum from bull 122
,, In domestic animals, 2, 3, 7, 235
,, In game in N. Rhodesia, 233, 234
,, In wild *G. morsitans*, 240, 243
,, Measurements 252
,, Morphology 251
,, Pathogenicity 253
,, Transmission 254
T. rhodesiense 245
,, Development in *G. morsitans* 273
,, Development in *G. morsitans* influenced by meteorological conditions 204
,, Distribution of 185
,, In domestic animals ... 235
,, In game in N. Rhodesia, 233, 234
,, In *G. morsitans* in nature 215, 240, 242
,, Measurements of ... 27, 218
,, Pathogenicity of 223
,, Posterior nuclear forms in rats 101

PAGE

T. rhodesiense Reservoir of 214
,, Transmission experiments with *G. morsitans*..... 187
,, Transmission experiment with *O. moubata* 301
T. theileri in domestic animals 3, 7
T. tragelaphi, sp. nov..... 269
,, Diagnosis..... 269
,, In game in N. Rhodesia 234
,, Morphology 269
,, Transmission 270
T. ugandae, sp. nov. 308
,, In domestic animals 3, 4
,, Posterior nuclear forms ... 3
T. vivax In domestic animals, 2, 3, 6, 235
,, In game in N. Rhodesia, 231, 233, 234
,, In rabbits 563
,, Measurements of 246
,, Morphology of..... 245
,, Pathogenicity of 247
,, Transmission 247
Trypanosoma, sp. (*? montgomeryi*), diagnosis 263
,, sp. (*? montgomeryi*) in domestic animals ... 235
,, sp. (*? montgomeryi*), morphology..... 261

PAGE

Trypanosoma, sp. (*? montgomeryi*), measurements 262
,, sp. (*?montgomeryi*), pathogenicity 261
,, sp. (*? montgomeryi*), transmission 263
Typhoid, prevention in the Panama Canal Zone 138
Vomiting sickness in Jamaica 377
Wallace, A. F. Attempt to transmit *T. rhodesiense* by means of *O. moubata* 301
Wallace, A. F., and Lloyd, Ll. Experiment to ascertain whether Tabanids transmit trypanosomes in nature 299
Yellow fever in Jamaica 467
,, ,, Prevention in the Panama Canal Zone 136
Yorke, W., and Blacklock, B. *Trypanosoma vivax* in rabbits 563
Yorke, W., and Blacklock, B. The probable identity of *T. congolense* (Broden) and *T. nanum* (Laveran)... 603
Yorke, W., Lloyd, Ll., and Kinghorn, A. Final report, Luangwa sleeping sickness commission 183

INDEX OF SPECIES NEW TO SCIENCE

PAGE

Aedimorphus quinquepunctata..... 598
Chrysoconops nocturnus 593
Culicelsa centropunctata 599
Diplococcus jamaicensis 465
Glossina severini 331
Heptaphlebomyia kingii 601
Herpetomonas stratiomyiae 609
Kingia maculoabdominalis 597

PAGE

Micrococcus castellanii 533
Mucidus nigerrimus 591
Reedomyia sudanensis 595
Trypanosoma ignotum 263
,, *multiforme* 254
,, *nigeriense* 356
,, *tragelaphi* 269
,, *ugandae* 308

FIG 1

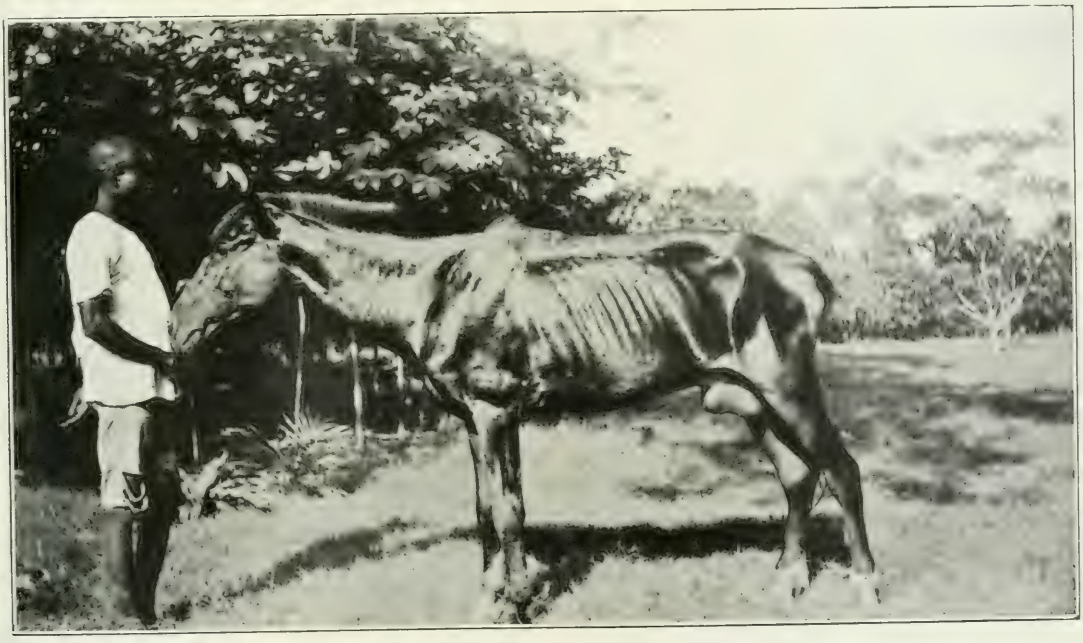

FIG. 2

C. Tinling & Co., Ltd., Imp.

FIG. 3

FIG. 4

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FIG. 5

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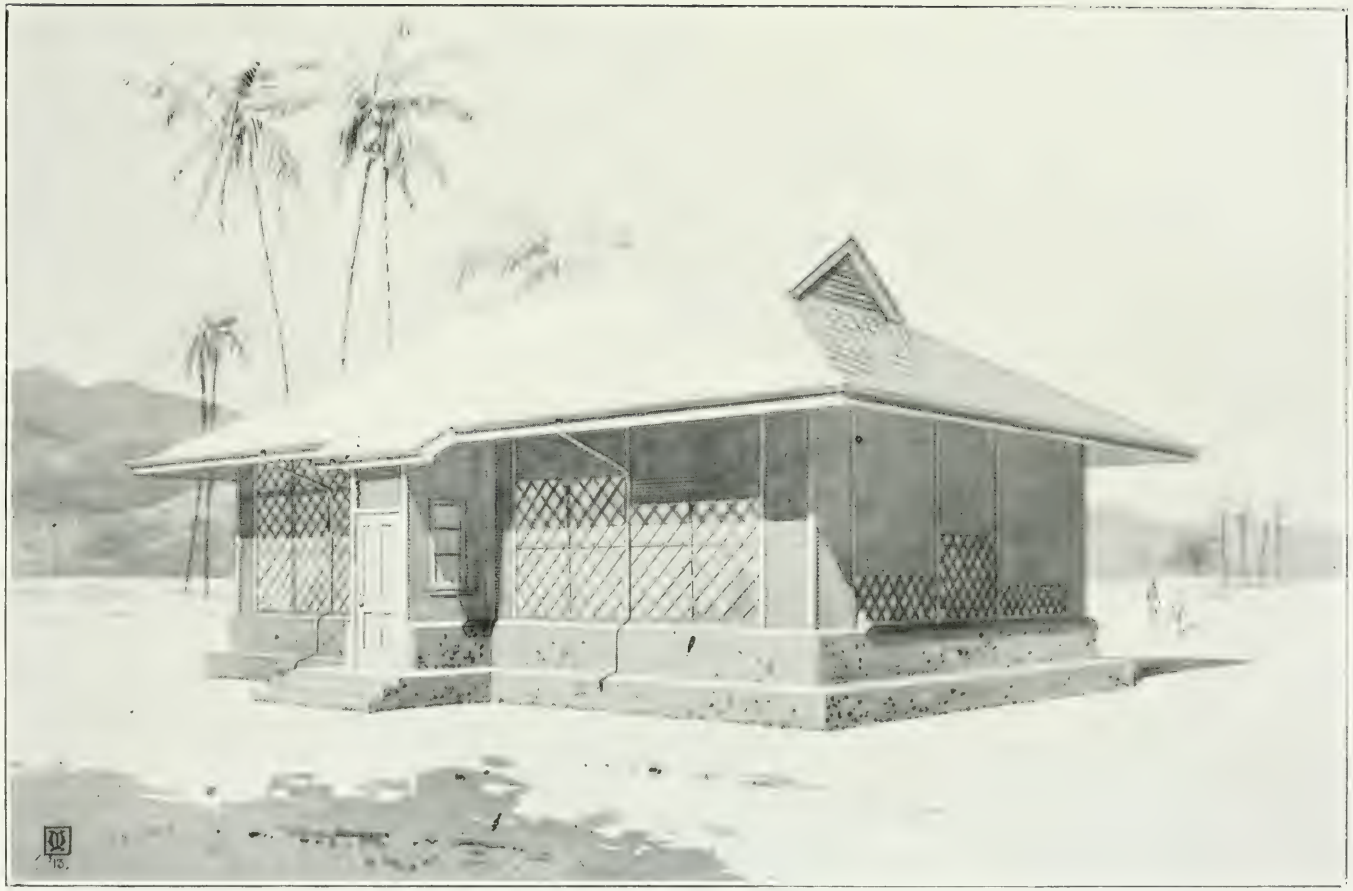

Designed by T. F. G. Mayer

NEW MOSQUITO-PROOF AND STORM-PROOF HOUSE FOR THE TROPICS

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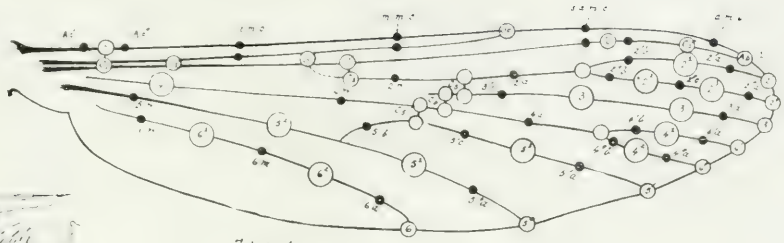

Fig. 1.

Fig. 4.

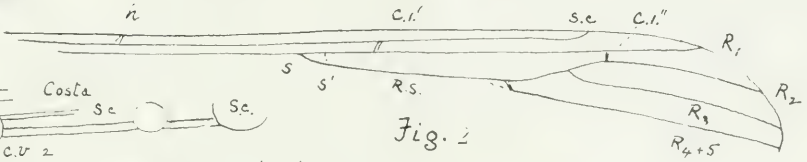

Fig. 2

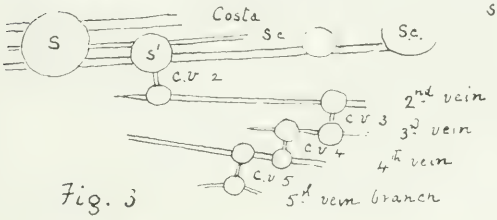

Fig. 3

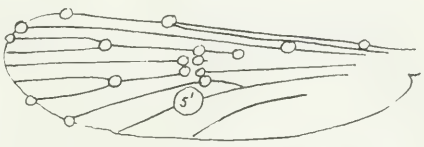

Fig. 9. Myzomyia. (a).

Fig. 5. Anopheles.

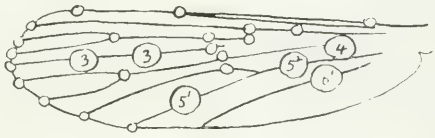

Fig. 10. Myzomyia. (b).

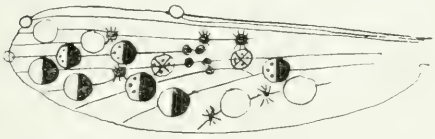

Fig 6. Myzorhynchus.

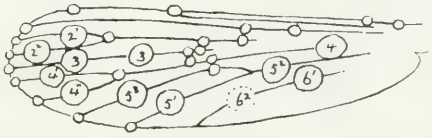

Fig. 11. Pyretophorus. (a) and (b).

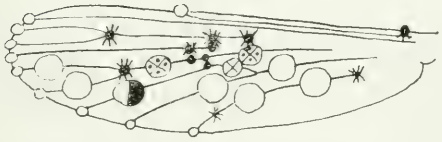

Fig 7 Cyclolepteron.

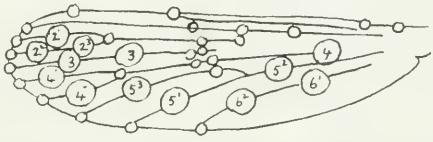

Fig. 12. Myssorhynchus, Cellia etc

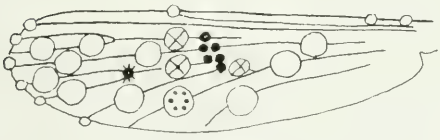

Fig. 8. Pataqiamyia

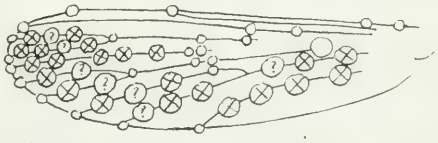

Fig. 13. Neomyzomyia.

O = nodal.
◯ = Internodal
⊗ = additional

✳ = scale aggregations.
◑ = dark scaled beneath.
◌ = mixed dark and light scales

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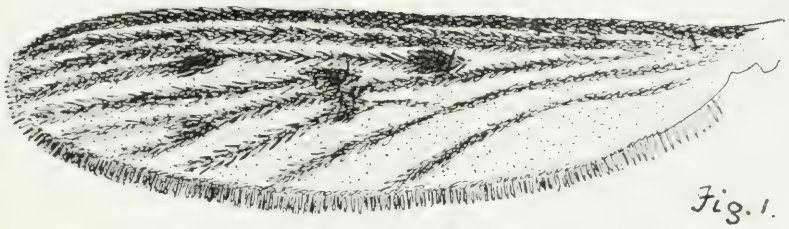

Fig. 1.

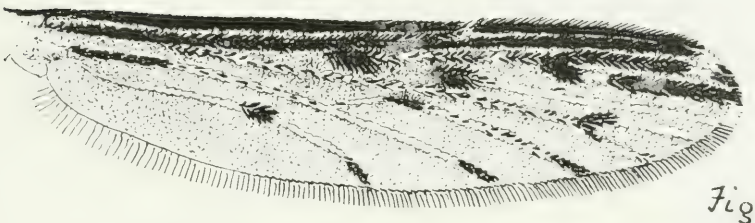

Fig. 2

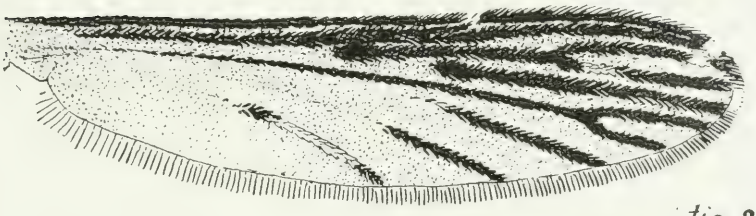

fig. 2.a.

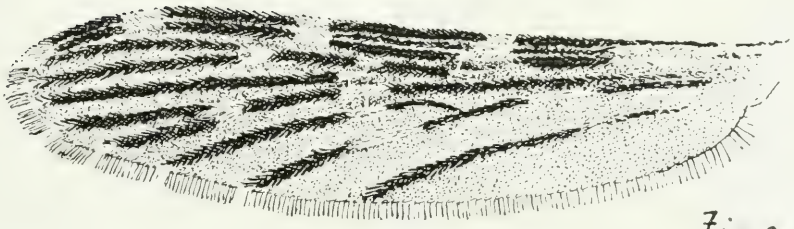

Fig. 3.

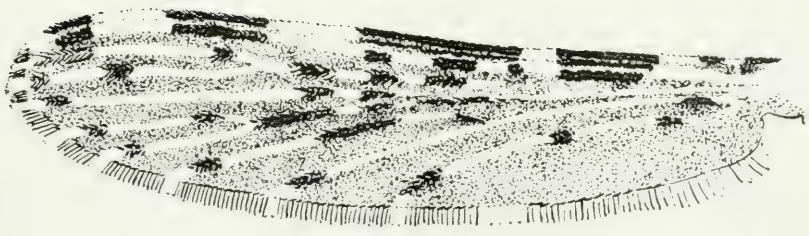

Fig. 4.

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FIG. 1

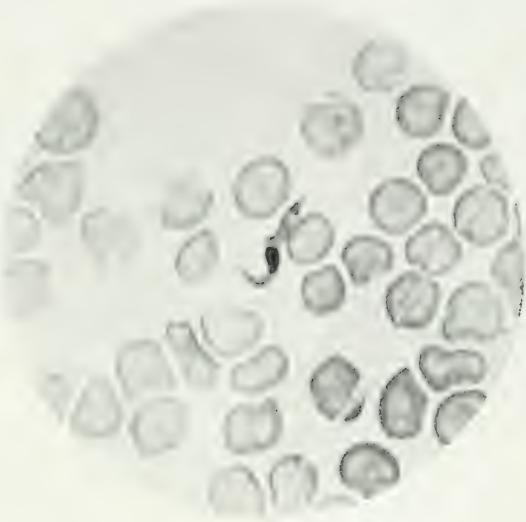

FIG. 2

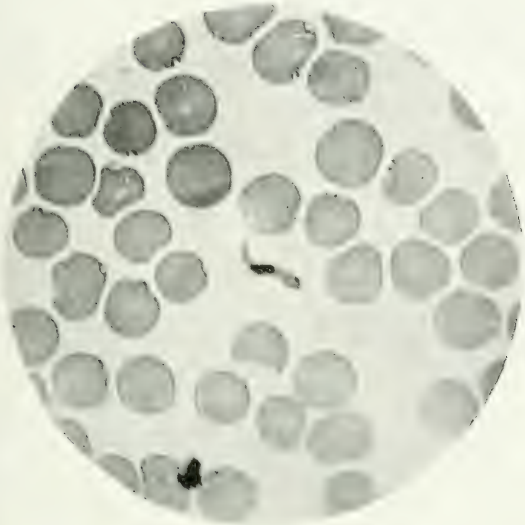

FIG. 3

FIG. 4

FIG. 5

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FIG. 7

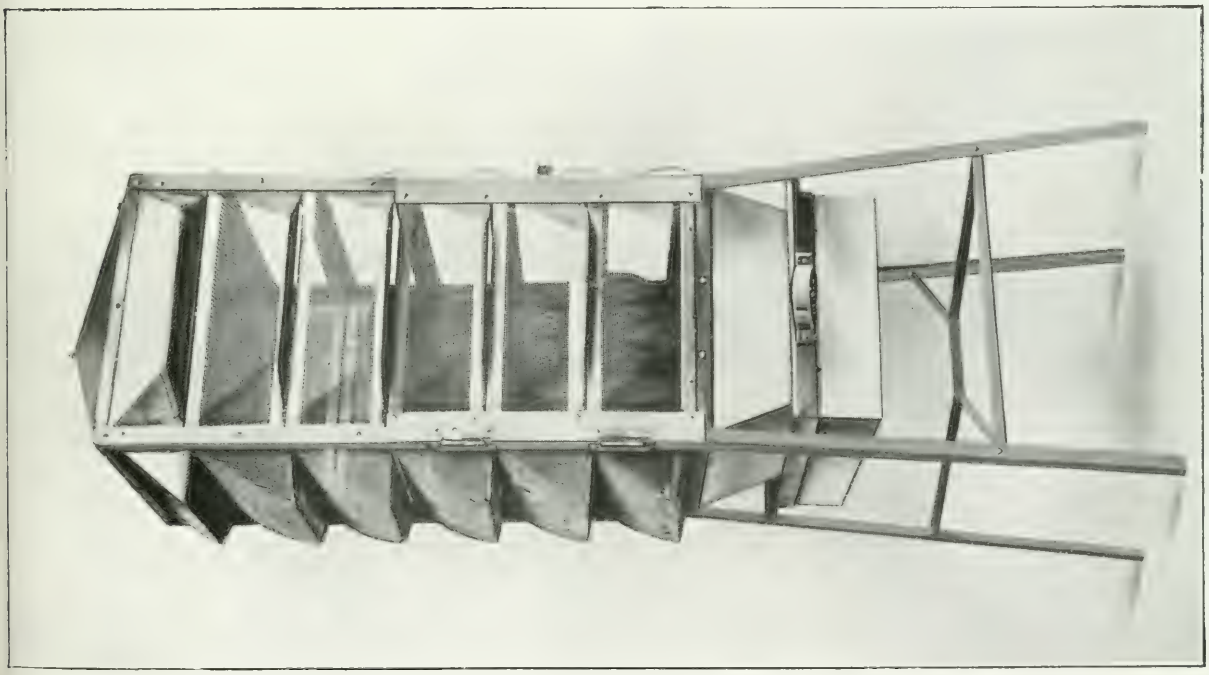

FIG. 6

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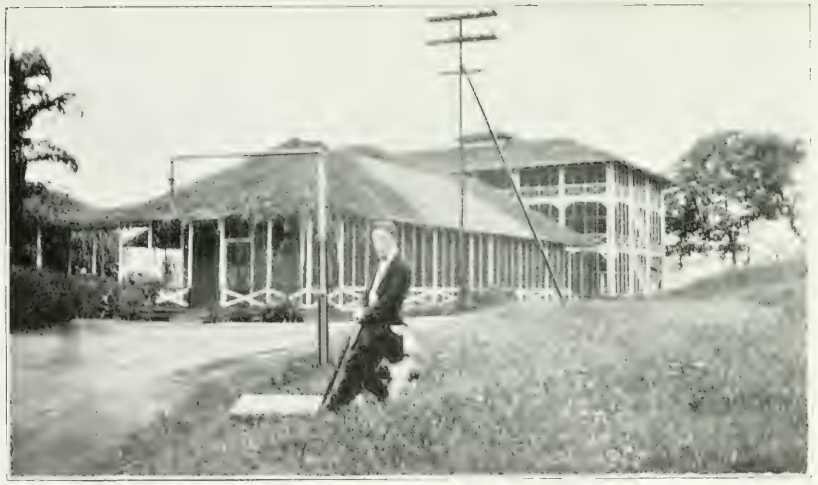


FIG. 6

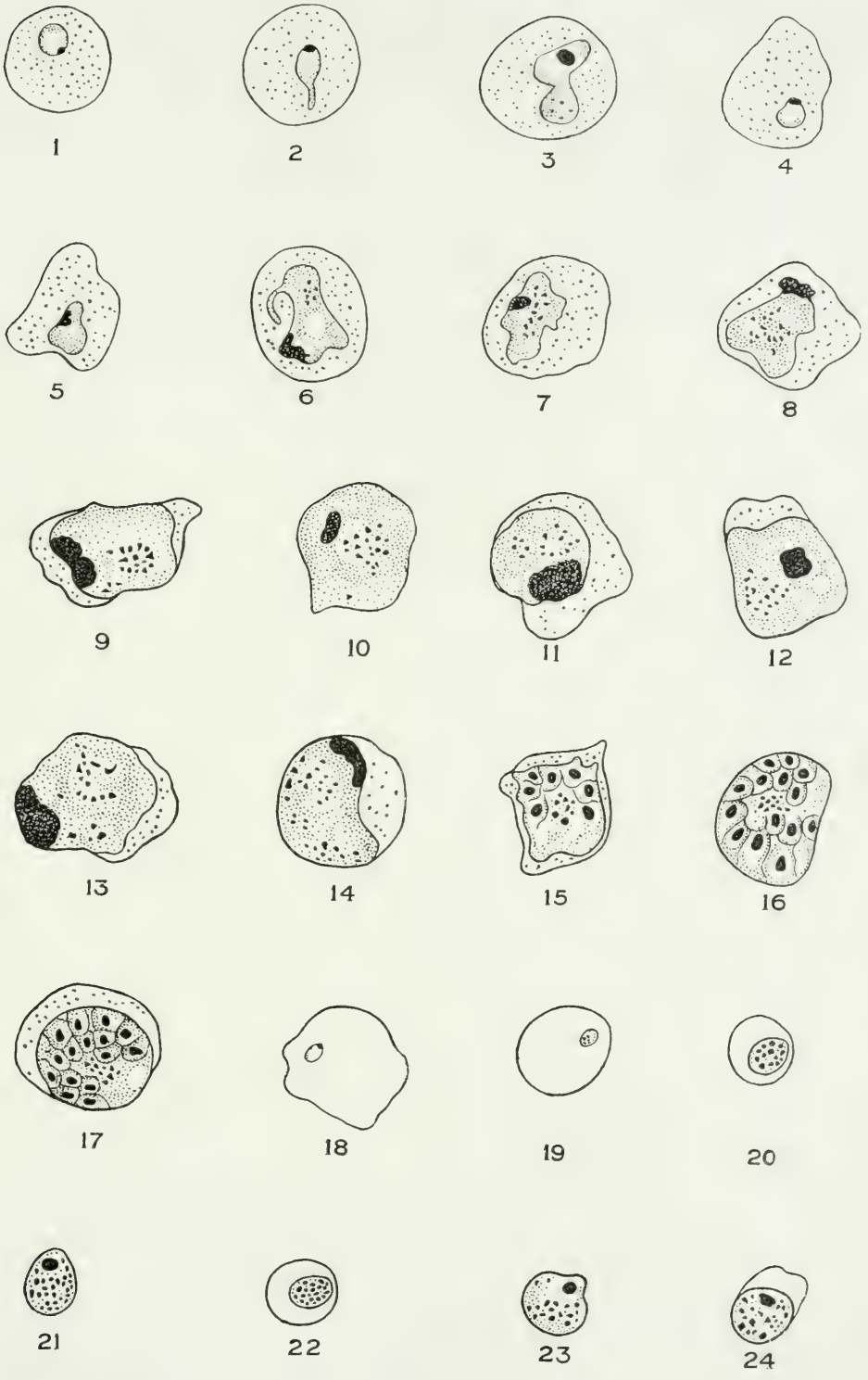
FIG. 7

FIG. 8

FIG. 9

D. Thomson, photo.

C. Tinling & Co., Ltd., Imp.



H. B. Fantham, del.

CULTIVATION OF *PLASMODIUM VIVAX*

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TRYPANOSOMA RHODESIENSE

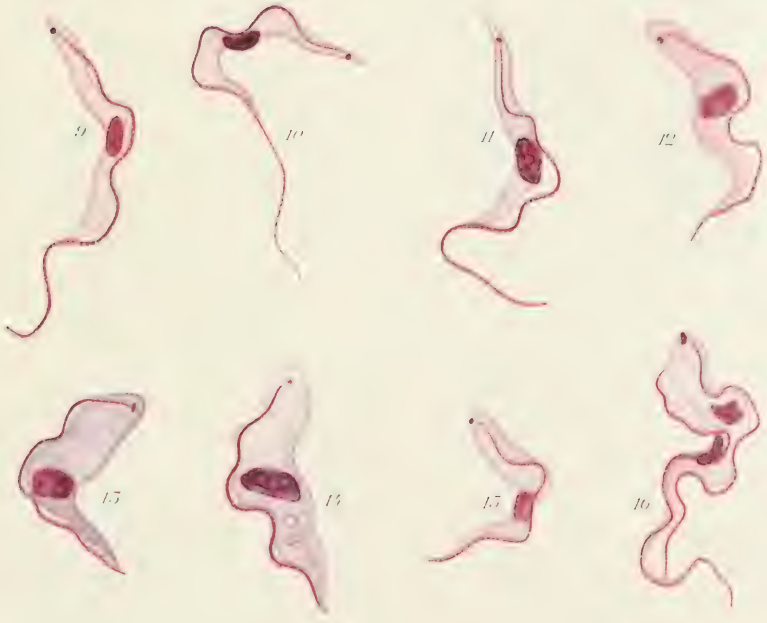
A. M. Brookfield, del.

TRYPANOSOMA VIVAX

A. M. Brookfield, del.

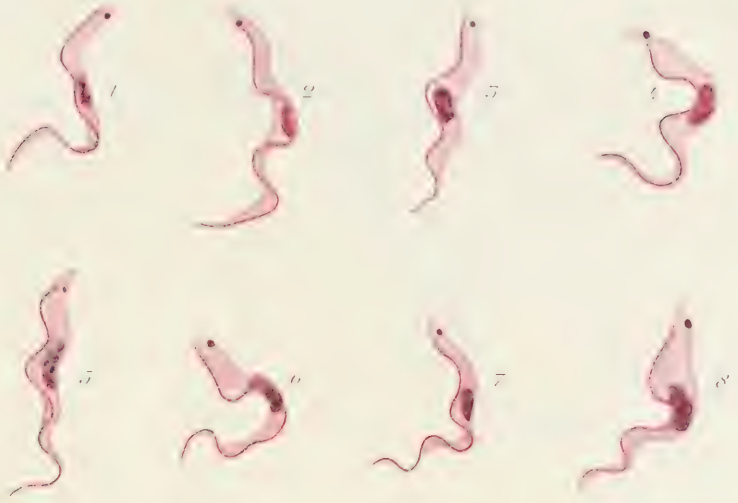
TRYPANOSOMA NANUM

TRYPANOSOMA PECORUM

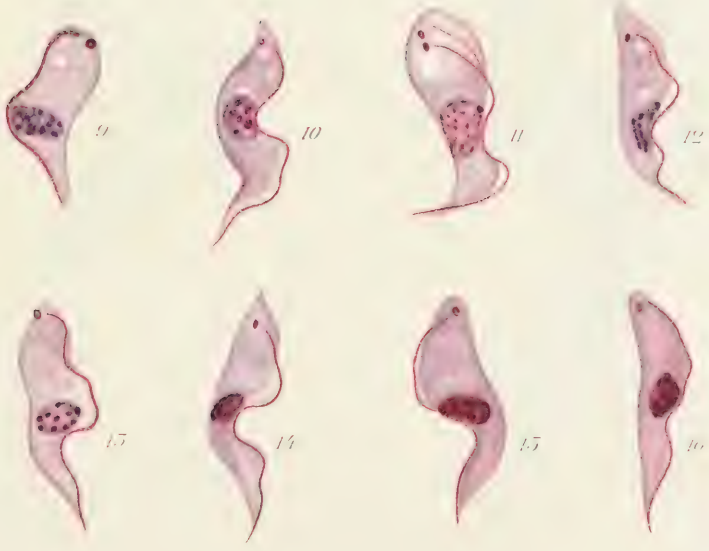


TRYPANOSOMA MULTIFORME

A. M. Brookfield, del.



TRYPANOSOMA IGNOTUM



TRYPANOSOMA MONTGOMERYI

A. M. Brookfield, del.

Firenze, Lit. A. Ruffoni, Piazza S. Croce 21

TRYPANOSOMA TRAGELAPHI

A. M. Brookfield, del.

A. M. Brookfield, del. Lemercier & Koffi

TRYPANOSOMA RHODESIENSE
IN GLOSSINA MORSITANS

FIG. 1. Pupal habitats of *Glossina morsitans.*

FIG. 2. Pupal habitats of *Glossina morsitans.*

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FIG. 3. Pupal habitats of *Glossina morsitans*.

FIG. 4. Pupal habitats of *Glossina morsitans*.

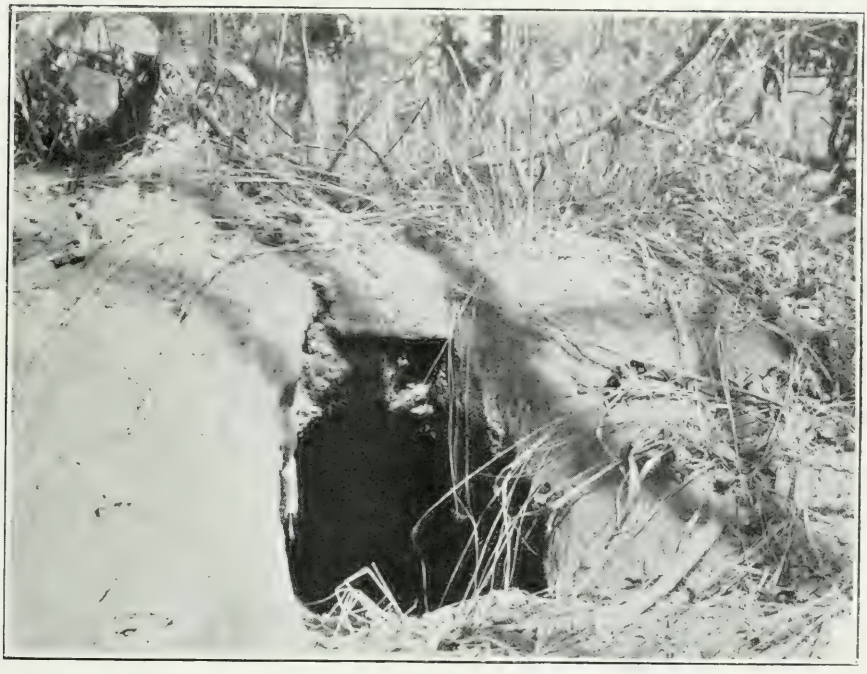

FIG. 5. Pupal habitats of *Glossina morsitans*.

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ROOT OF *IPOMOEA*.

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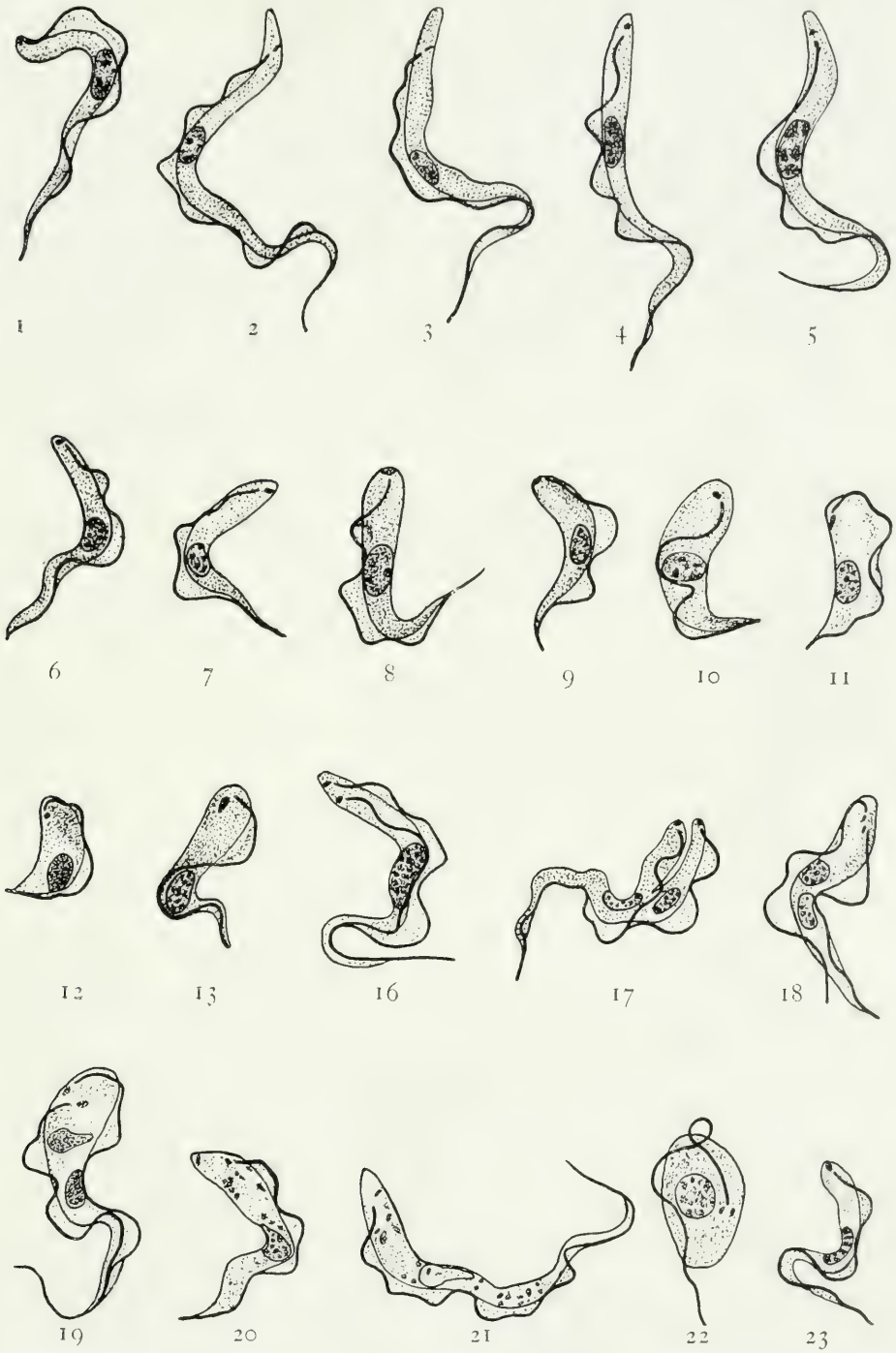

TRYPANOSOMA NIGERIENSE

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FIG. 1

FIG. 2

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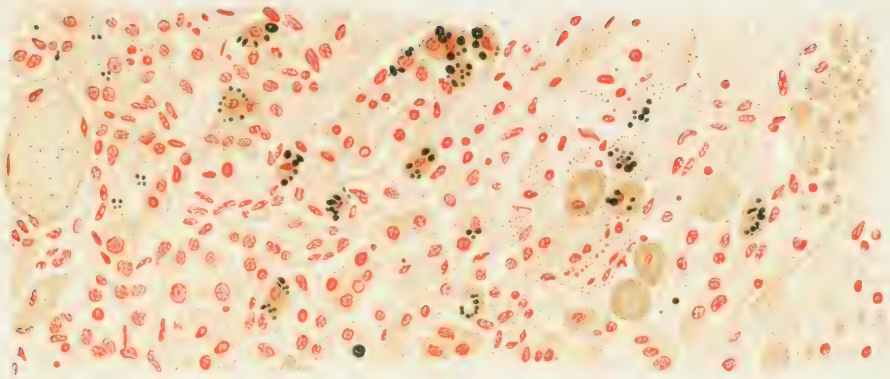

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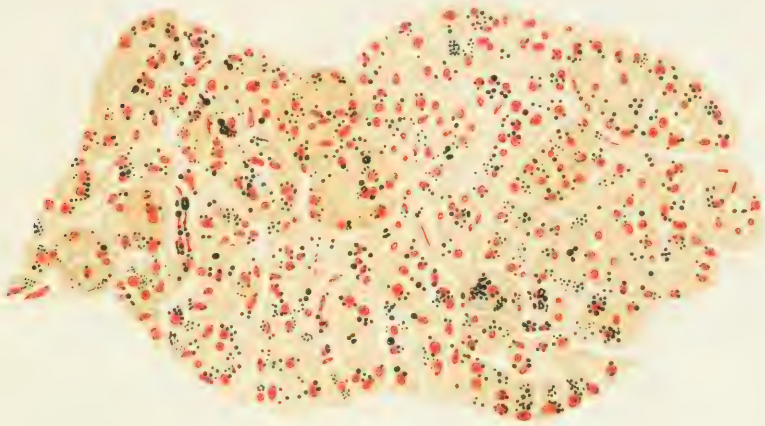

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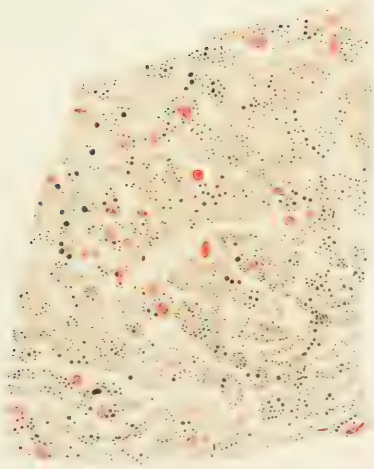

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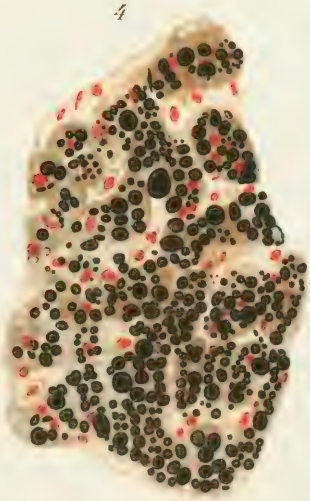

Margrethe Seidelin pinx.

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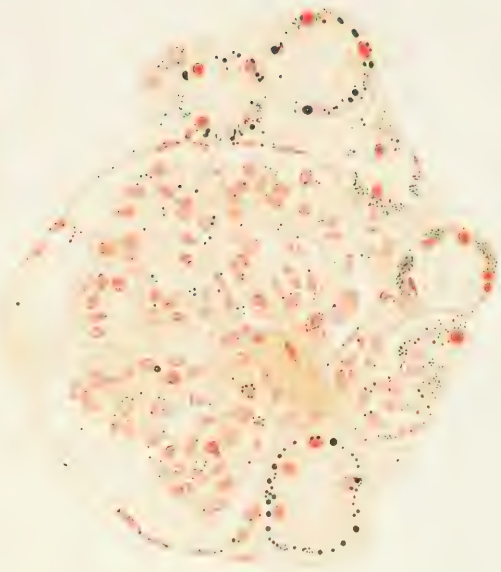

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4

Margrethe Seidelin pinx.

PLATE XXXII

1

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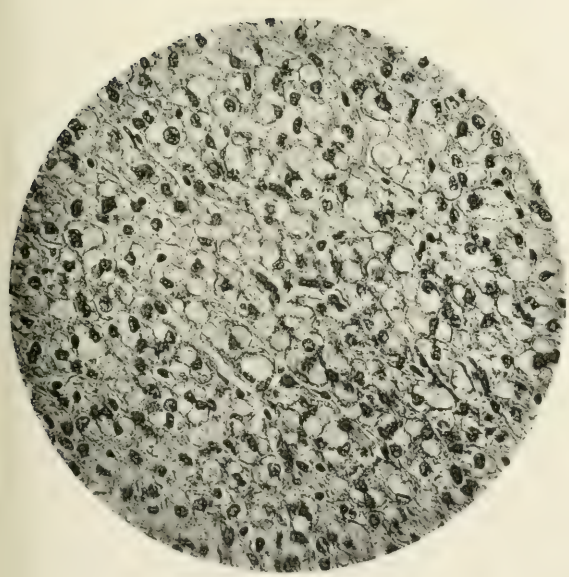

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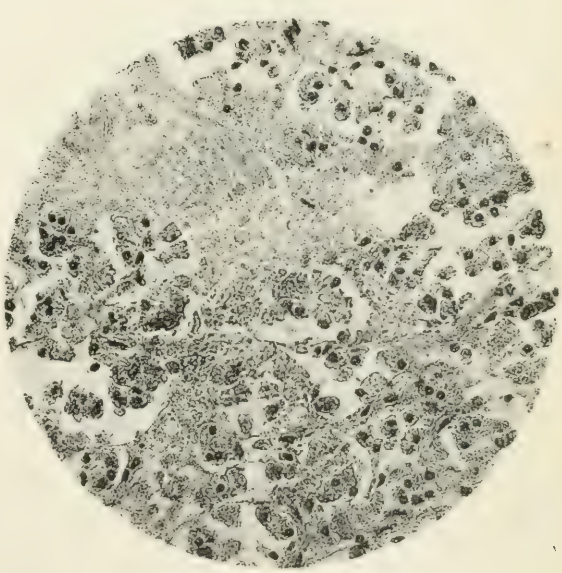

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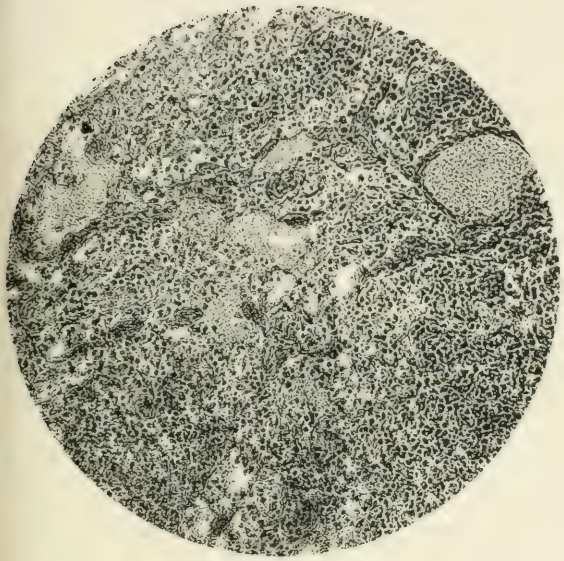

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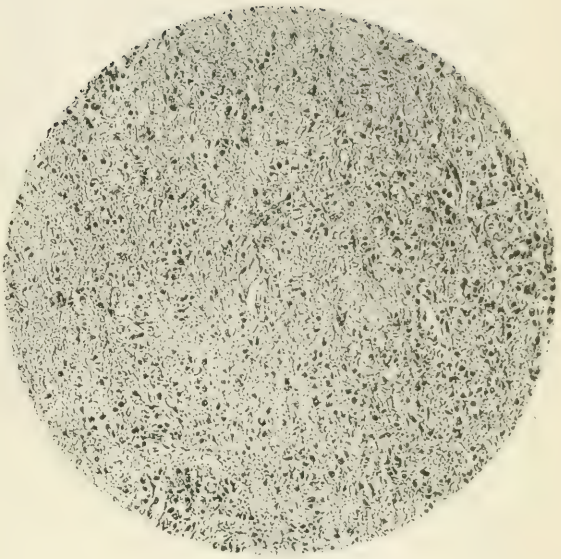

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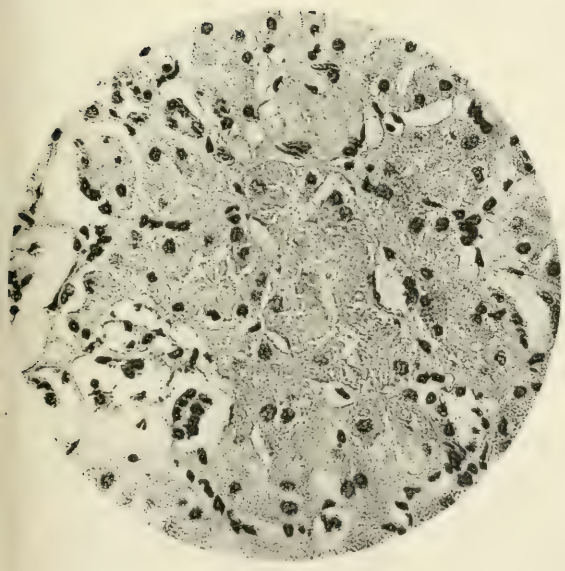

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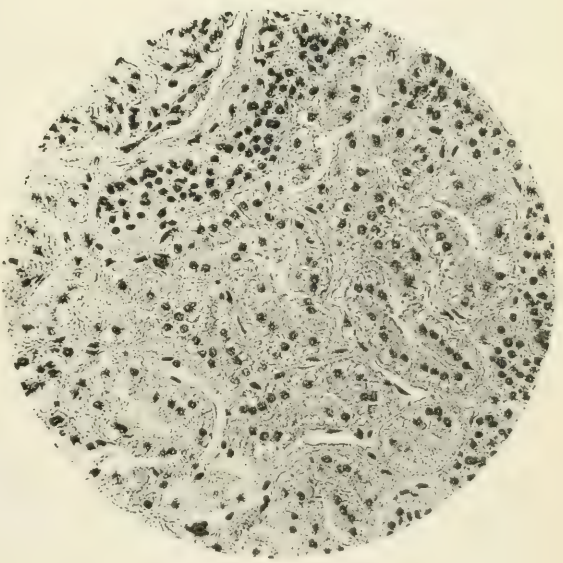

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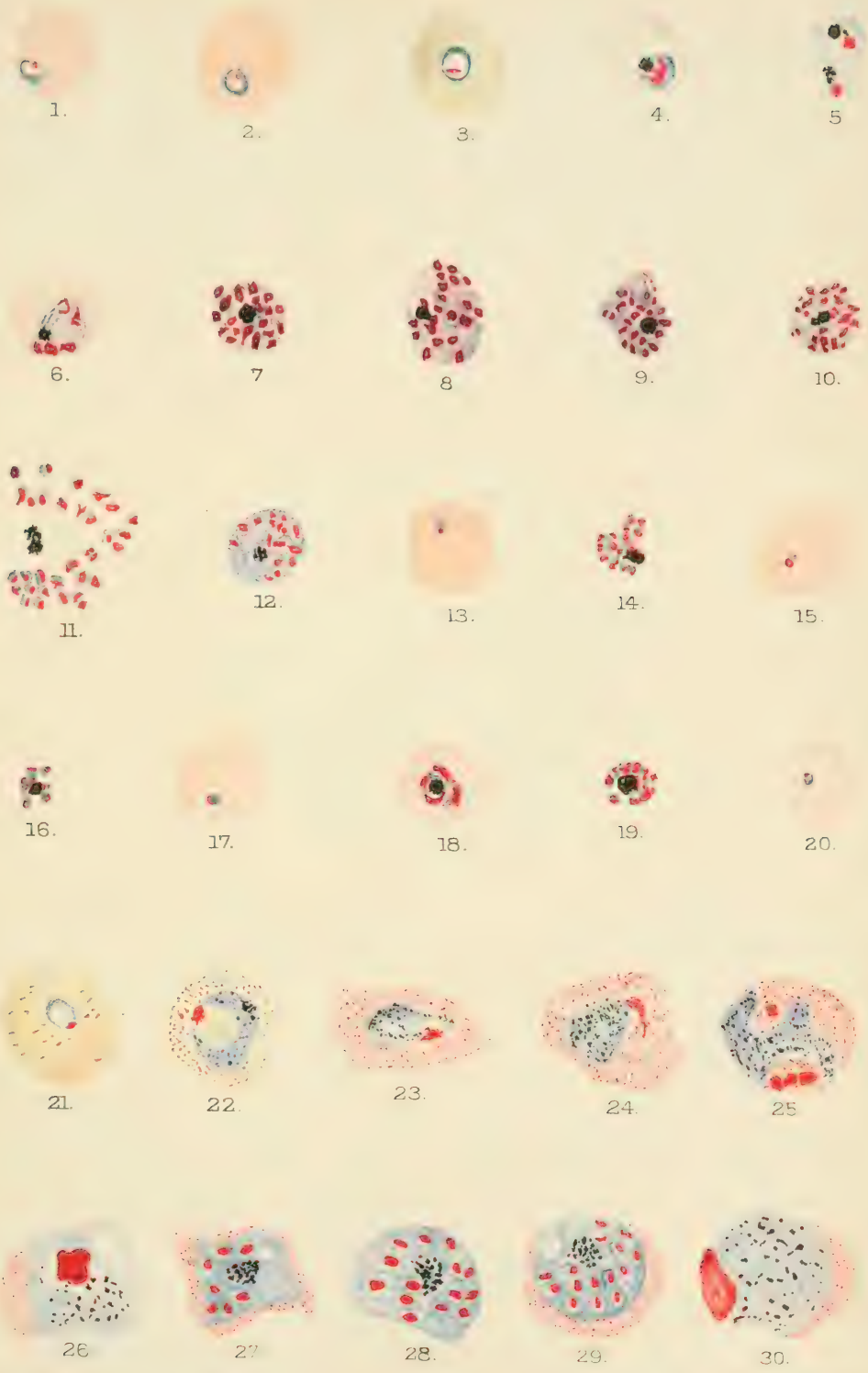
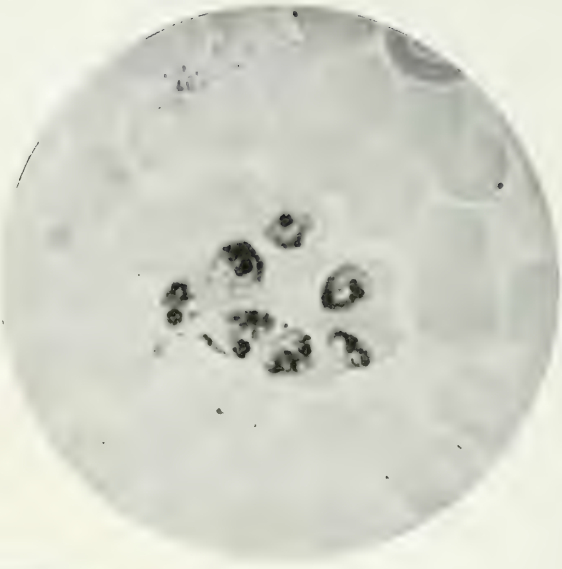


J. G. Thomson & D. Thomson, del.

Firth lith. et imp.

CULTURAL FORMS OF MALARIAL PARASITES.

1



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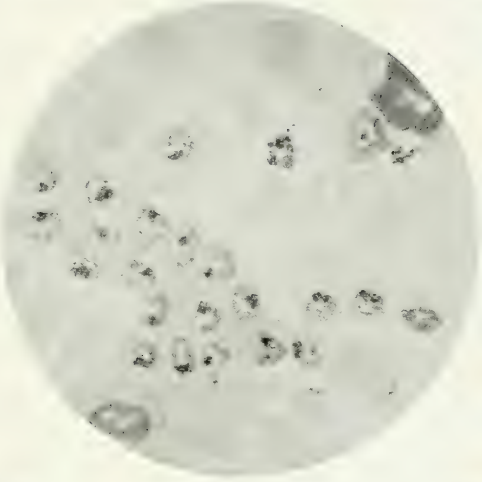

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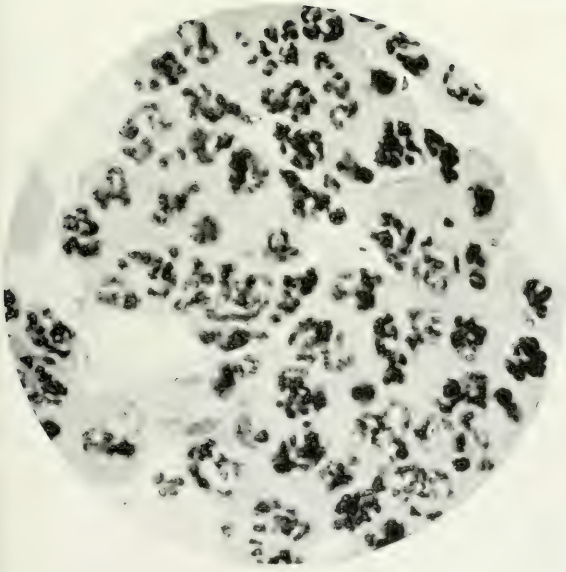

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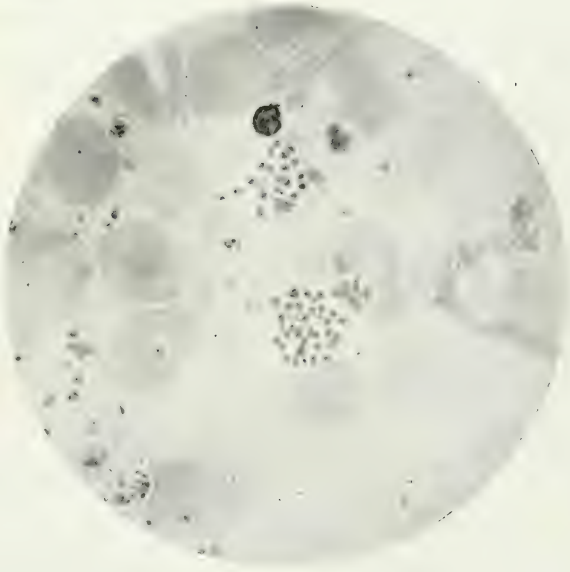


4

SPORULATION OF THE MALIGNANT TERTIAN PARASITE

Micro photographs by Thomson, J. G. and D.

C. Tinling & Co., Ltd., Imp.

THE TRICHONOCARDIASES

Firenze Lit. A. Roffoni Piazza S. Croce 20

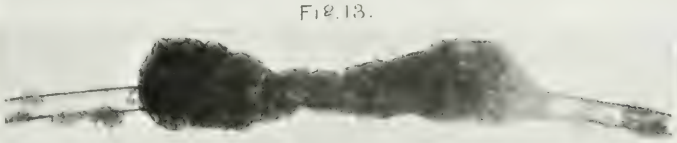


Fig. 13.

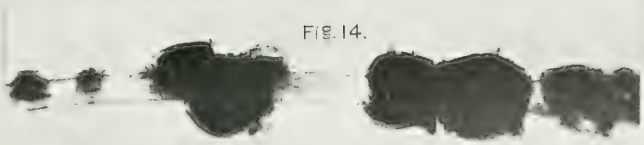
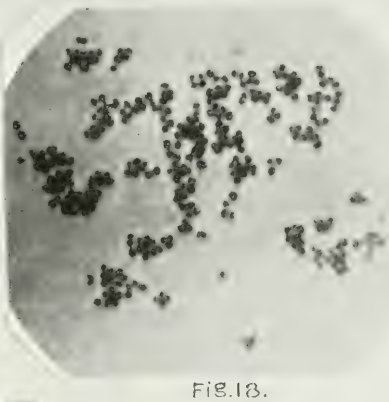


Fig. 14.

Fig. 15.

Fig. 16.

Fig. 17.



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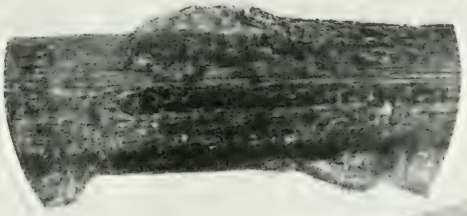

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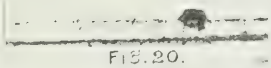


Fig. 20.

Fig. 21.

Fig. 22.

THE TRICHONOCARDIASES

C. Tinling & Co., Ltd., Imp.

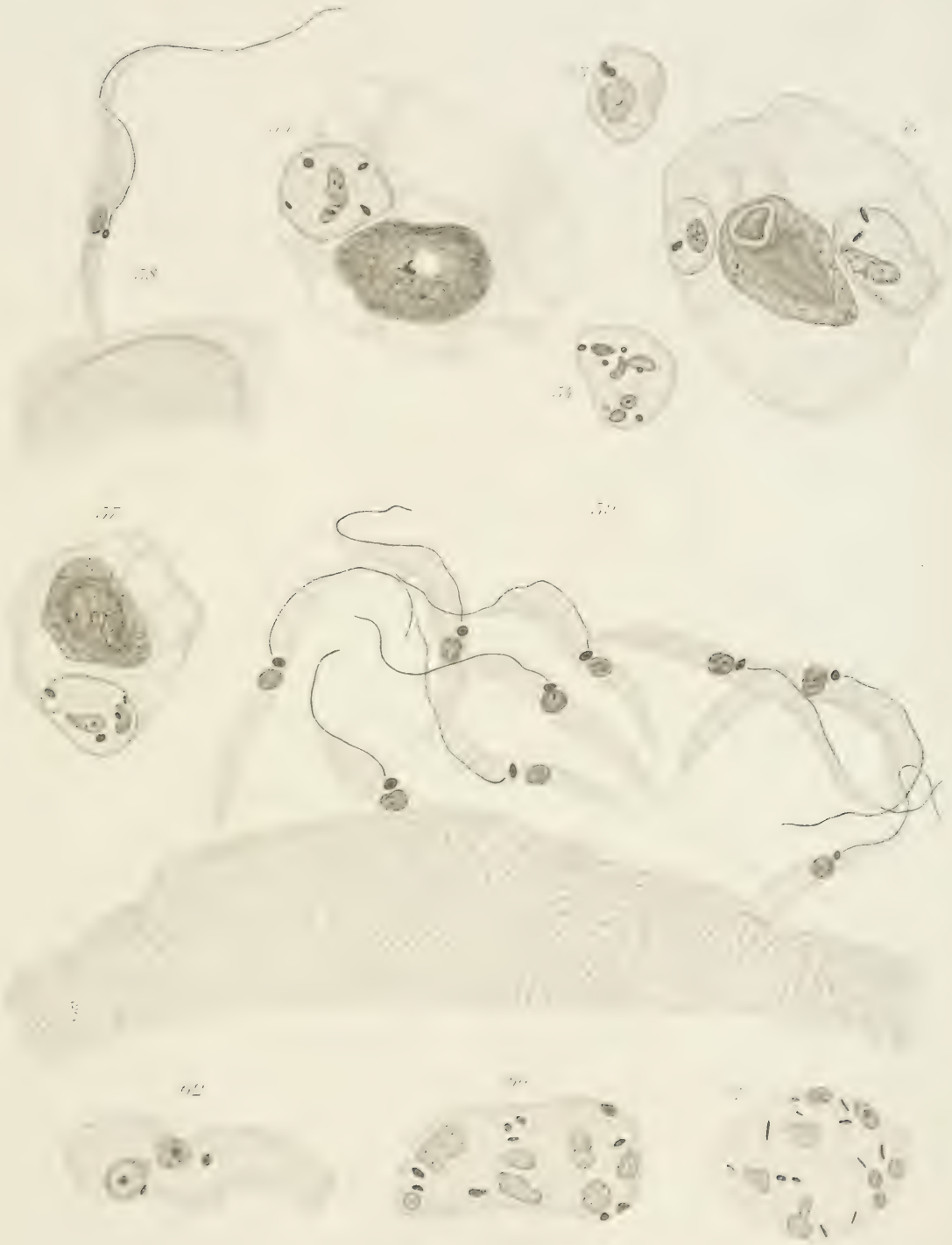
CRITHIDIA HYALOMMAE

W. R. O'Farrell del.

Firenze, Lit. A. Ruffoni, Piazza S. Croce 20

CRITHIDIA HYALOMMAE

W.R.O'Farrell del.



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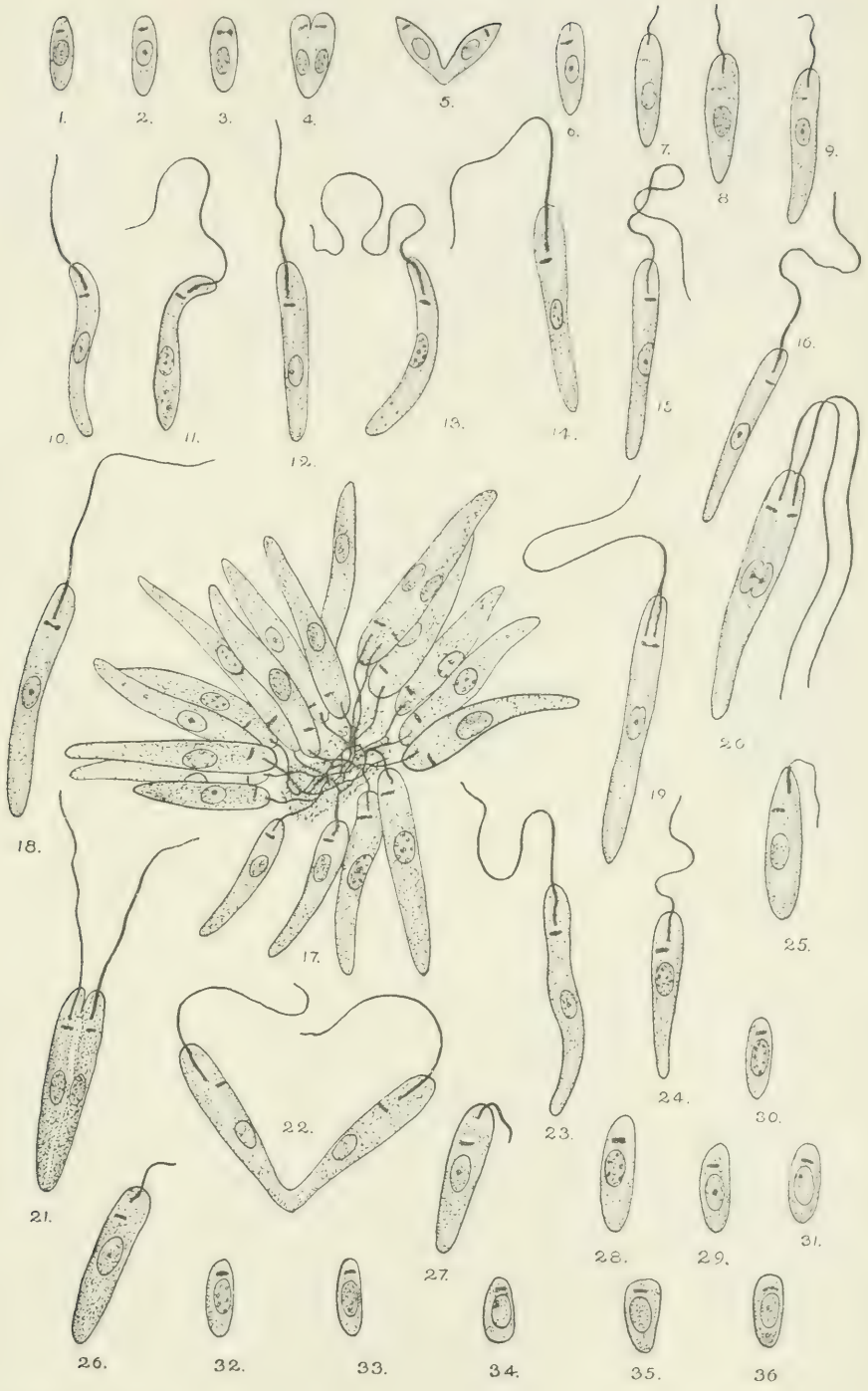

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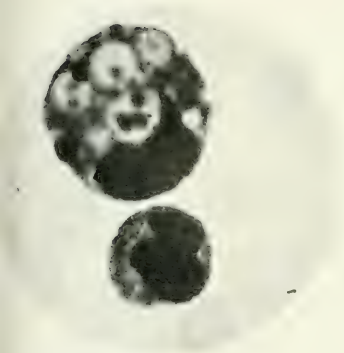

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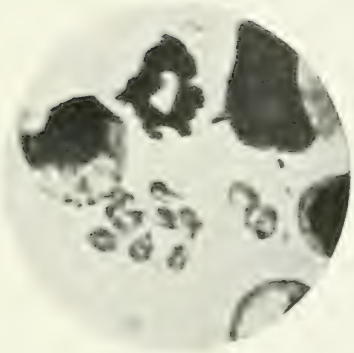

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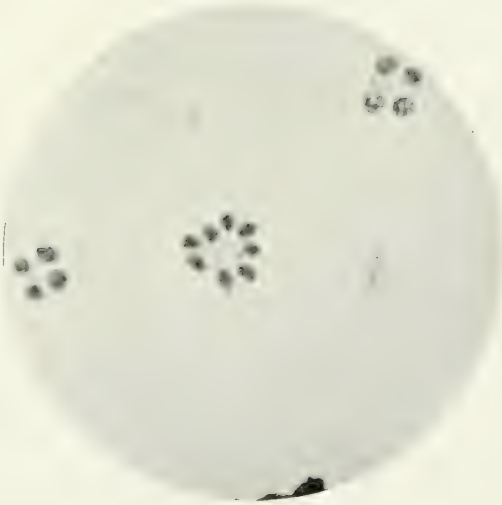

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